

Limits to SALT...

EUPHORIA at the news that President Ford had achieved something in the way of a step forward in arms control during his recent meeting with Mr Brezhnev in Vladivostok has been short lived. Although it is certainly encouraging to know that Dr Kissinger has been able to persuade Mr Ford of the importance of keeping SALT high on the list of priorities, the expectations for the next round of talks are likely to be limited and, paradoxically, talk of a long control period (up to 1985) is bad rather than good news. The signs now are that SALT-2 will restrain both superpowers to do only what they intended to do in any case and will allow them ten years before they need to take up the running on limiting arms again.

SALT-2 froze for five years (1972-77) the total number of ballistic missile launchers at 1,710 (United States) and 2,350 (Soviet Union). Of these not more than 710 and 950 respectively could be submarine-based, and the conversion of land-based launchers from 'light' to 'heavy' character was prohibited. The figures were arrived at by little more than an examination of the *status quo* in 1972 and a belief that, if the numbers were disparate, quality made up for quantity. The ultimate in quality was, of course, to put many warheads on each missile, and the impossibility of satellite inspection of MIRVs ruled out any control of their extent.

Two years later the process of MIRVing is proceeding rapidly, with much of the United States fleet both at sea and on land already converted, and Soviet intentions (if lagging) perfectly obvious. Proposals for SALT-2 now include multiple warheads in the list of constraints, not, as far as one can gather, because of dramatic improvements in satellite inspection but presumably because it is considered economically desirable not to go the whole way on MIRVing.

Perhaps the most interesting development, however, is the agreement to trade off concessions. In return for acknowledgement that in SALT-2 strategic bombers will be included in the count of delivery vehicles (an American concession since their bombers out-number Soviet ones 3:1) the Soviet Union will not urge inclusion in the treaty of American forward-based systems. It seems that the total number of vehicles each side will then agree to keep to is about 2,500—a figure that the Soviet Union is already at and that the United States is close to. And 1,300 of these vehicles on each side would be fitted with MIRV, legitimising a total of nearly 2,000 more MIRVed missiles than exist at present!

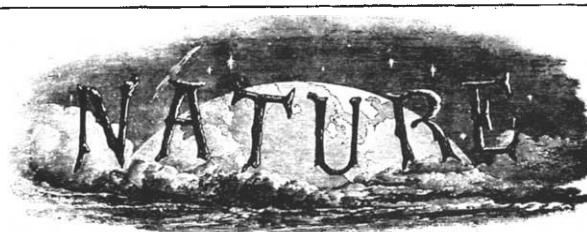
The quantitative plateau on which both sides will happily take a rest is hardly likely to pose many problems of unemployment for the military-industrial complex. Accuracy and manoeuvrability have long since replaced size as the growth points of the missile field. Undoubtedly the next step is the MARV, or manoeuvrable re-entry vehicle, which can pursue a complex course

to its target, and no doubt sometime in the late 1980s when these have an accuracy of 10 metres or so, the superpowers will start talking about constraints on them.

... and UNESCO

ANY discussion on the status of Jerusalem is bound to be charged with emotion, and anyone who chooses this particular instant to raise questions of the maintenance of the cultural heritage of the Old City must be assumed to be intent on making the maximum of mischief out of a complex situation. The sponsors, then, of the UNESCO resolution inviting the Director General to cut off assistance to Israel for its 'persistence in altering historical features' and undertaking excavations which endanger monuments 'subsequent to its illegal occupation of the city' were not acting out of purely archaeological concern. Whatever the rights or wrongs of the 'cultural' case against Israel there could hardly have been a more divisive time to raise this issue, and the sponsors, Arab and communist countries, are guilty of hypocrisy if they believe that the rest of the world will see their initiative as other than vulgarly political.

If the sort of moralistic criteria that they are applying to Israel were applied uniformly then Britain should be condemned for retaining the Elgin marbles, the Soviet Union and Czechoslovakia for severe restrictions on academic freedom, the United States for its past oppression of the Indian community and so on. Smart diplomats could rip apart UNESCO within months. But perhaps they need the contributions from the big boys more than they do from Israel.



THE TRANSIT OF VENUS

THE long-anticipated Transit of Venus took place yesterday morning; and already has the first instalment of news from distant observers arrived. The Astronomer Royal has been good enough to inform us that Col. Tennant's observations at Roorkee, India, have been quite successful; 100 photographs have been taken. He also telegraphs, at the moment of going to press, the gratifying intelligence that the micrometric observations near Cairo and Suez, and the photographic observations at Thebes have entirely succeeded.

At the last meeting of the Astronomical Society the Astronomer Royal gave an account of the final arrangements of the English parties, which do not vary much from those we stated some time ago. Messrs. Green have arranged for one of their outgoing ships to pass near Kerguelen's Land, with a view of picking up intelligence and telegraphing it from Melbourne.

The southern stations occupied by the American, French, and German parties leave no doubt that the Halleyan method will be extensively employed.

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CETI: put not your trust in beacons

D. R. Bates, of the Department of Applied Mathematics and Theoretical Physics, Queen's University, Belfast, argues that it would be unjustified to devote resources to the interstellar communication problem on the assumption that there is a neighbouring technological civilisation which continuously operates a beacon signal suited to us.

THOSE active in promoting interstellar communication, for example through the \$10,000 million Project Cyclops, have faith that a neighbouring technologically advanced civilisation, X, would continuously operate a suitable beacon signal that, in addition to showing our civilisation (a technological novice) the star to which to send the acknowledgement signal, would carry other information. Most favour the beacon being omnidirectional because such a beacon by embracing all possible respondents within range, would avoid the contact-time difficulty.

People are heedless to the needs of their descendants as their exploitation of the Earth's resources demonstrates. No terrestrial government would support a costly beacon if it were advised that a response (of debatable benefit) would be unlikely to be received for 200 years. In the case of a government on X, I shall quixotically suppose that the corresponding period is normally 1,000 years. This millennium criterion is a key factor. It would be imprudent for us to incur expenditure on the assumption that X, if indeed it exists, has a succession of governments with a multi-millennium criterion or with a sustained resolve to pass knowledge to alien species on distant planets.

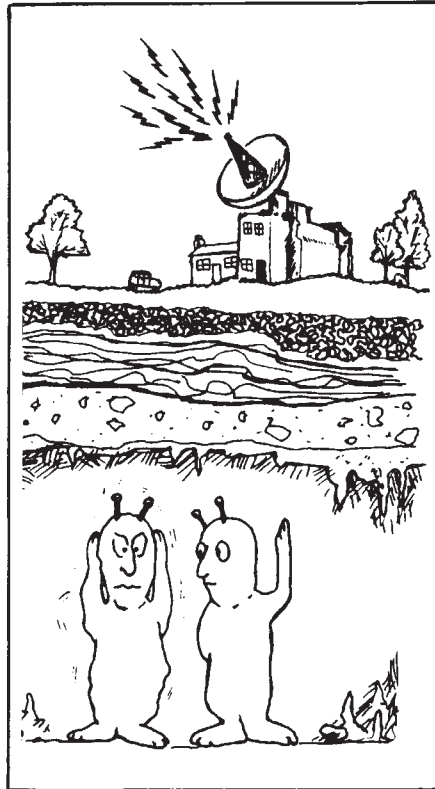
On the millennium criterion the government of X would only finance a beacon if the probable distance to the closest potential respondent were expected to be less than 500 light years. From the spatial distribution of stars it follows that the number of potential respondents in the Galaxy would have to exceed the critical number

$$N_c \approx 5 \times 10^4 \quad (1)$$

Drake's equation gives

$$N = RL \quad (2)$$

N being the equilibrium number of technological civilisations in the galaxy, R their annual rate of emergence and L their mean lifetime in years. The widely quoted estimate for R , 0.1 per



year, cannot be much too low. It may well be much too high: thus it rests on the dubious assumption that the abundant M and K-type stars are amongst the hosts and on the dogmatic assumption that the unknown probability of life developing on an apparently suitable planet is unity. Young technological civilisations (up to several times as old as us with age measured from the release of nuclear energy) are clearly so few compared with N_c that they would be dismissed as potential respondents on the millennium criterion.

Some may survive adolescent dangers and conceivably have lifetimes on the stellar evolutionary scale. Perhaps a sufficient fraction do so to make L long enough (at least millions of years) for N_c to be exceeded. Even granted this, a doubt remains. Ancient civilisations would have explored virtually all of science; they would have sophisticated historical records and rich cultural heritages to study. Many may lose interest in possible signals and even in astronomy, leaving the number in the communicative phase less than required.

Suppose that X has hitherto been isolated. Doubtless signal detection has been thoroughly tried: it has the attraction that its initiators might themselves have the satisfaction of witnessing success. Scientists of X could only

guess the probable distance to the closest potential respondent. Some might ask a government to finance a beacon project. For a government to spend unnecessarily on the basis of a guess would be strange. My quixotic supposition is that it does so provided the project satisfies the millennium criterion. In addition to the costs of constructing the beacon and associated power station the costs of maintaining and replacing them arise. Thought would therefore be given to the length of time for which transmission would be justified.

The number of advanced civilisations within the range of the beacon fluctuates around its equilibrium value. Such civilisations emerge at the annual rate

$$R_B = R/N_c \approx 2 \times 10^{-5} R \quad (3)$$

which is very slow. Hence the chance of getting a response would not be significantly increased by signalling for the full millennium rather than just for several decades. Several decades would suffice for the beacon to be noticed by any interested advanced civilisation within range and for much information to be carried by its signal (though whether or not this would reach another civilisation would be a dispiritingly open question).

If no replies came by the end of the millennium scientists on X would conclude that no potential respondents were within range. To increase the beacon's range ten-fold would necessitate increasing its power a hundred-fold. Scientists wishing this might attempt to find support for signalling efforts which at best might be acknowledged between 1,000 and 10,000 years later. We would be prodigal to divert resources from promising research on the assumption that they would succeed.

If replies to the first beacon came, the triumph would not make it much easier to persuade a government of X to finance a second. A better case could be made for financing a brief beacon project (covering hitherto unexplored stars) by a government of one of the respondents not already in contact with other civilisations.

Should a communication cell once be established, its radius might expand at a speed comparable with that of light until the entire Galaxy were occupied. For this to be possible the total number of civilisations in the communicative phase would have to exceed N_c , which is large. Because of the slowness of the emergence rate given

by equation (3), it would be imprudent for us to proceed on the assumption that a government of a civilisation belonging to the hypothetical communication network would finance a prolonged beacon project aimed merely at hastening contact with any relatively recently developed civilisation rather than leaving the initiative to the newcomer as the one having the greater incentive and the easier problem.

The inference that it would be irresponsibly optimistic to assume that civilisations operate beacons for more than minute fractions of their lifetimes in the communicative phase has an important consequence. It follows im-

mediately from Drake's equation that relatively few beacons can realistically be expected to co-exist. A proper allocation of research effort cannot ignore that we are hence probably much farther from the nearest beacon than the beacon is from its intended respondents. The acute difficulty this causes is greatly aggravated by another factor.

The communication techniques of a civilisation initially improve rapidly but improvement cannot continue indefinitely. Instead the rate of improvement must decrease and eventually become vanishingly small. Presumably all civilisations which survive long enough

reach a common level of technical attainment, and presumably most beacons are designed by and suited to those at or near this common level. A comparison between Projects Ozma and Cyclops shows strikingly how rapidly our techniques are still improving. The pattern of our development can scarcely be such that Project Cyclops, though far ahead of Project Ozma, is close behind what may ultimately be achieved. Yet, unless the pattern is indeed such, we must be technically much too backward for it to be worth our while engaging in the basically extremely formidable task of searching for signals. □

international news

PERSISTENT reports of the arrest, torture and execution of doctors and other health workers have been filtering out of Chile ever since Salvador Allende's government was overthrown by the armed forces last year. Although the military junta has consistently denied such charges, most conspicuously in a full-page advertisement in the *New York Times*, an investigation by three biomedical scientists with support from the Federation of American Scientists (FAS) has now accumulated considerable evidence that left wing members of the medical profession have indeed been a prime focus for particularly brutal reprisals since the junta seized power.

Most of their findings simply back up many previous allegations of brutality, but the report breaks new ground with its analysis of why doctors in particular were singled out for harsh treatment and it also has some disquieting things to say about the Colegio Medico—the Chilean equivalent of the British Medical Association.

First, the Colegio played an active part in Allende's downfall by organising two damaging strikes, one in 1972 and the second immediately before the coup, and it issued two demands for Allende's resignation. After the armed forces seized power and began rounding up Allende's supporters, however, the Colegio did nothing to defend the rights of any of its members, even though its own code of ethics states that it is obliged to defend accused physicians. As Dr Leonard Sagan of the Palo Alto Medical Clinic, who led the investigation, puts it: "the Colegio took almost a brutal delight in the overthrow (of Allende) but it had no compassion whatsoever for its members who were arrested".

Reprisals against Chilean doctors

by Colin Norman, Washington

In particular, the report notes that one doctor, Carlos Shuster, wrote a letter to the Rector of the University of Chile suggesting that all physicians be classified into three groups "those above suspicion, those with leftish sympathies and those beyond rehabilitation". That letter "seems to have suggested to authorities a general system for classification of health workers into A, B, and C lists", the report suggests, and those lists later served as a basis for arrests and other reprisals.

Local responsibility for compiling the lists fell to directors of hospitals and chiefs of service, the report states, and "while we were unable to obtain direct evidence, it seems probable that officials of the Colegio Medico, on the national and local levels, were involved in preparing lists of physicians considered not only dangerous but also politically unacceptable". It adds, however, that only in the Medical School of the University of Chile "did we hear, from the dean, a clear distinction between ideological allegiances and participation in violence. The dean asserted that, at least within his jurisdiction, this distinction has been respected."

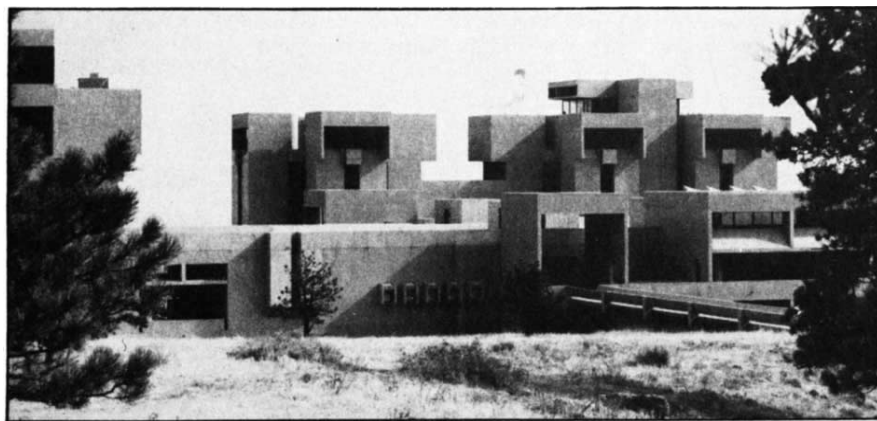
Appearance on a list had a variety of consequences. "In some cases it appears that certain physicians were marked for death", the report states; others were arrested and several have

been barred from working for the health service—which is tantamount to denying them a job at all.

The investigating team says that it has "good evidence" of 176 physicians who have been suspended from the health service, and it has a list of 109 physicians who had been imprisoned up to June this year. Dr Sagan said last week, however, that the figure for total arrests of doctors is probably nearer 300. In addition, some 250 doctors have fled the country since the coup.

As for reports of executions, the team arrived in Chile with a list of 28 doctors believed to have been shot, but several of the reports turned out to have been unfounded. They found good evidence, however, that at least fifteen doctors have died since the coup, and "of those deaths for which we have relatively reliable information, death occurred shortly after arrest in the month of September". The most common excuse given by the authorities was that prisoners were shot while trying to escape, but the report states that such a story is implausible at best.

Finally, the report notes that allegations of torture are widespread, but "are difficult or impossible for foreigners to substantiate in a charged political environment". Nevertheless, the investigating team repeatedly heard two disturbing allegations about the source of the methods of torture used. First, a report that a group of Brazilians were brought to Chile to train the Chilean military was "widely heard" and, second, "there are frequent references to an American training camp in Panama where South American military officers are indoctrinated in techniques of resisting torture, thereby providing technology on the information of torture".



Trends in meteorology

by John Gribbin

THE National Center for Atmospheric Research (NCAR) in Boulder, Colorado, has just issued its annual report for 1974. The NCAR was set up in 1960, and is run by the University Corporation for Atmospheric Research (UCAR), a private, non profit corporation whose 44 members are universities and other institutions with doctoral programmes in the atmospheric sciences. The operation of the NCAR is supported by the United States National Science Foundation; in practice, this means that the NSF runs the NCAR for the UCAR on a contractual basis. The Center is unique—there are only four other national centres run by the NSF and all of those are centres of astronomy. Indeed the NCAR is itself responsible for the High Altitude Observatory (HAO) but this is not as odd as it might seem, since that observatory carries out research on the physics of the Sun and the solar terrestrial environment, areas of astronomy which seem to have great relevance to problems of the behaviour of the Earth's atmosphere.

Public, and indeed journalistic, attention has focussed on centres like the NCAR lately because of the increased awareness of the problems of climatic change. But as the new annual report shows, this kind of work has been somewhat out of the mainstream of research at the NCAR. That situation may now be changing but more traditional aspects of atmospheric research are clearly still at the forefront as yet.

Topics covered in the past year include research into storm forecasting, the investigation of the interaction of aerosol particles with liquid and ice constituents of clouds, and the airflow and moisture budget of a hailstorm. Some of the work on mechanisms of hail growth highlights just how great the problems of understanding all the workings of our atmosphere are—some theories of hail formation postulate that hail growth begins with the freez-

ing of large supercooled water droplets; others argue that it begins with ice riming around individual ice crystals. Studies at the NCAR show that, for Colorado storms, growth from the ice phase is indeed dominant; but Soviet workers report great success with the alternative model. As the authors of the report put it “geographic and en-

Cloud research

PROFESSOR Jim Megaw, of York University, Toronto, says that as far as man's influence on climate is concerned “the time for descriptive work is past”. Like many other people he is greatly concerned about the effect of freon on the ozone layer and believes that its use as a propellant in spray cans should be stopped. Another problem which needs urgent attention is the growth of industrial clouds from the smoke and gas emitted by factories; here, Megaw is at least on the trail of a technique which would make possible the destruction of man's influence on the environment at source.

It seems that laboratory experiments on cloud formation have from time to time been hampered because the ‘clouds’ refused to form in the cloud chambers. The blame for this has now been laid at the door of the pure ion exchange water used by the experimenters. There is something in ion exchange water which can inhibit the formation of mist, clouds or fog, and it seems very likely that the elusive ‘something’ is an amine (one of the same family of compounds as those which give fish their characteristic odour). Megaw is now trying to identify the exact compound involved, which is effective in tiny quantities. If it can be pinned down, it could be added to industrial products to inhibit formation of industrial mist and fog. Although Megaw did not mention the possibility of use in aircraft, this would also seem an ideal way to reduce or remove the hazard of increased cloud cover produced by high flying jets.

environmental differences may provide some explanation of the discrepancy”, but if both theories can be correct, depending on circumstances, then a real understanding of hailstorms is far from being at hand.

If that is the case with something as seemingly simple as a hailstorm, the job of those working on climatic modelling might seem impossible. But progress is being made, at the NCAR and other centres. One study of the effect on surface temperatures which might result from changes in stratospheric aerosol or ozone concentrations brought about by operating a fleet of supersonic transport (SST) aircraft seems likely to fan the flames of the SST controversy once more. The effect of the increased stratospheric aerosols on the NCAR model is a reduction in surface temperature by 0.25 K, whereas the effect of the nitrogen oxides emitted by the hypothetical SST fleet is a rise in surface temperature by as much as 1 K. In the first case, increased reflection of solar heat dominates; in the second there is a net increase in absorption. These studies offer no easy solution to the question “is an SST fleet environmentally safe?” but since a change in surface temperature of 0.1 K is considered by the experts to promise significant climatic effects it does seem that the question should at least be asked once again before SST operations begin on a large scale.

Studies of the feedback between temperatures and cloud cover also show how climatologists must solve the chicken-and-egg problem. We hear a lot about how increased cloud cover can cause more reflection of solar heat and a fall in surface temperatures. On the other hand, preliminary results of studies now continuing at the NCAR indicate that there is a positive feedback between changes in surface temperature of the sea and the cloud cover.

The solar studies of the HAO also extend to include work on Skylab, through the Apollo Telescope Mount, and the Executive Director of the NCAR, John Firor, sees studies of the Sun as of increasing importance to problems of climate and atmospheric modelling. Such statistical correlations as there are between solar phenomena (notably the sunspot cycle) and terrestrial weather have not been explained by any satisfactory physical model, but they have encouraged a new look at the solar influence. Indeed, now that the astrophysicists have been forced, by the absence of detectable solar neutrinos, to accept that they do not know all about the workings of the Sun there is room for climatologists to question just how constant the ‘solar constant’ radiation reaching the Earth is. □

WHEN the submersible Alvin (based at the Woods Hole Oceanographic Institution) returned this summer from one of the most successful undersea research programmes ever undertaken—the joint French-American exploration of a section of the Mid-Atlantic Ridge—it faced an uncertain future. Because of a chronic shortage of funds for submarine research, Woods Hole officials were then saying privately that Alvin, which is reckoned to be the best of the few research submersibles operated in the United States, would be mothballed.

But last week three federal agencies announced that they have joined forces to keep Alvin operating for at least three years. The National Oceanic and Atmospheric Administration (NOAA), the National Science Foundation (NSF) and the Navy will each put up \$300,000 in 1975. They will renegotiate the funding levels for 1976 and 1977 on an annual basis, but it is estimated that about \$1 million will be found for each of those two years.

The new funding arrangement, apart from being welcome news to Alvin's research crew, is something of an experiment. The NSF has supported Alvin's work in the past on a project-by-project basis, but according to one NSF official, "we would now like to see what the scientific demand will be with a stable funding level". Alvin's scientific programme for next year is now being worked out.

● Three years ago, members of the American Chemical Society (ACS) opted for a decisive shift in the ACS's activities when they elected Alan Nixon as the society's president. Nixon conducted a vigorous election campaign (which itself was without precedent in the society's affairs) based on the promise that he would make the ACS more responsive to the professional needs of its members. And he has been followed by two like-minded presidents—Bernard Freidman this year and William Bailey next. The upshot is that the ACS has greatly expanded such activities as manpower surveys, public information and Congressional liaison, and it has even recently set up a loan fund to help with the legal expenses of its members who are in dispute with their employers.

But there are signs that the society may now be opting for a change back to its more traditional style of leader. Last month the members elected Glenn T. Seaborg, a former Chairman of the Atomic Energy Commission and a nuclear chemist with impeccable academic credentials, to be president of the ACS in 1976, whereas a candidate added to the ballot by chemists

who generally supported Nixon's policies for the ACS came in third. Moreover, Nixon himself failed in a bid to be elected to the ACS Board of Directors.

Even though Seaborg's election is generally viewed as a break with the tradition established in the past few years, it should be noted that he at least paid lip service to the need for the ACS's professional programmes in his election statements.



Washington seen

by Colin Norman

● The General Accounting Office (GAO), an investigative arm of the United States Congress, has carried out a survey of security systems at nine nuclear power stations in the United States, from which it has concluded that they are probably ineffective in preventing reactor sabotage aimed at releasing large quantities of radioactive material into the environment.

The GAO's report, which was released last week by Senator Abraham Ribicoff, contends that "a security system at a licensed nuclear power plant could not prevent a takeover for sabotage by a small number—as few, perhaps, as two or three—of armed individuals". It adds that "such a takeover, particularly of a nuclear power plant near a large metropolitan area, could threaten public health and safety if radioactive materials were released to the environment as a result of successful sabotage".

In particular, the GAO found that in several of the plants it visited there were breaches of security regulations such as unlocked outside doors, lack of intrusion alarms, unarmed watchmen and unlit protected-area perimeters—security measures, in other words, which would pose little problem for an amateur burglar let alone an armed terrorist.

Be that as it may, there is considerable disagreement within the nuclear industry about the vulnerability of nuclear reactors to sabotage even if a saboteur did manage to gain access to

the reactor site. Consequently, the Atomic Energy Commission itself is sponsoring a number of studies aimed at defining the level of risk and the possible consequence of successful sabotage. The studies should be completed in July next year.

The GAO points out, however, that at least one part of a power plant seems to be both accessible and vulnerable to sabotage—the facilities used for temporary storage of used fuel rods.

In the United States, when fuel rods are removed from the reactor core they are placed in an uncovered pool of water near the reactor for cooling with the result that "they do not have the same degree of physical protection as that provided to the reactor core by the reactor containment vessel".

The spent fuel rods are only supposed to be stored at the reactor site for a short time before being packaged and shipped to reprocessing facilities. But the problem is that no commercial reprocessing facilities are yet operating in the United States, so that more used fuel is now on hand at power plants than normal. The GAO therefore recommends that interim security arrangements should be established immediately to protect this material.

● Having recently banned the manufacture of the pesticides aldrin and dieldrin, the Environmental Protection Agency (EPA) is now moving against two more widely used insecticides, chlordane and heptachlor. Last month EPA Administrator Russell Train announced that he intends to cancel the registration of those two pesticides—an action tantamount to removing them from the market—because they may be carcinogenic.

About 3 million pounds of heptachlor were used in 1972 on corn, vegetables, cereals, forage crops, seed crops and seed treatment, and about 16 million pounds of chlordane were used to control fruit and vegetable pests and for various household and commercial uses.

The EPA has decided to move against the pesticides because of a feeding study which has shown that heptachlor and its metabolite significantly increase the incidence of liver tumours in one strain of mice at the level of 10 parts per million in the diet, a feeding study with rats which showed up a tumorigenic effect at intake levels as low as 0.5 parts per million, evidence of embryotoxicity from both heptachlor and chlordane in some strains of rats and mice, and the discovery in hospitals in Atlanta of heptachlor metabolites in tissue taken from 10 stillborn infants.

Heavy water shortage faces India

from Narender K. Sehgal

IN less than two years' time, India is likely to find her heavy water requirements exceeding availability. So, what's new? Nothing except that, unlike other items, heavy water is a 'nuclear' material and its import from abroad may involve more than mere commercial considerations. (Heavy water is used as moderator and coolant in the Indian nuclear reactors.) In the wake of recent developments following India's explosion of a nuclear device, nuclear powers such as the United States, the Soviet Union and the United Kingdom along with Australia, Canada and West Germany are reported to have decided that no vital 'nuclear' materials shall henceforth be supplied to non-nuclear weapon countries unless they are subject to International Atomic Energy Agency (IAEA) safeguards under an agreement. It is not yet clear whether or not this implies that these countries will refuse to sell heavy water to India. The IAEA meeting at Vienna recently did not provide a clear cut answer.

Work is now in progress in India on three nuclear power projects, one each in Rajasthan, Tamilnadu and Uttar Pradesh. (One of the units of the Rajasthan project is already delivering power. At present, India has a small heavy water plant (14 tonnes annual capacity) at Nangal in Punjab. But four more plants with a total annual capacity of some 300 t are under construction at Kota (Rajasthan), Baroda (Gujarat), Talcher (Orissa) and Tuticorm (Tamilnadu). The production will reach maximum capacity in stages and only by 1978 will it near the 300 t mark—that is, if work goes on as planned.

According to Atomic Energy Commission calculations, India will have to find something like 200 t of heavy water from outside sources during 1976–77 and then again during 1981–83 when shortfalls will occur if no additional production capacity is installed.

In theory, design specifications provide 100% leak-proof heavy-water systems in reactors and after the initial loading (which means more than 200 t of heavy water for each of the reactors under construction in India) no more should be required. But in practice leaks invariably do occur during operation and arrangements are always made to recover the leaked fluid and upgrade it for reuse. However, some replenishing is always needed to make up for losses during the recovery process.

India is reportedly already negotiating with several countries in this con-

nection. If the countries mentioned above choose to enforce their decision, France may be the only alternative left. Then again, the question will hinge on whether or not France will be able to spare the amounts of heavy water required by India, after her own needs are taken care of. If it does not become possible to arrange the needed quantities of heavy water, the already bad power situation will worsen further as the nuclear power projects will either face delays or curtailments in power generation, or both—a price India may have to pay for her two 'sins': conducting a nuclear explosion and refusal to sign the nuclear non-proliferation treaty! □

Conservation golden jubilee

from Vera Rich, London

NOVEMBER 29, 1974 marked the golden jubilee of the All-Russian Society for Nature Conservation, an organisation which has grown over the years to a membership of some 26 million, and which has as its chairman no less a personage than the Deputy Minister of Land Reclamation and Water Economy of the Russian SFSR, N. G. Ovsyanikov. Accordingly, in addition to the official notice of the anniversary, which reported such diverse activities as reforestation, the protection of beavers, the study of the ecological effects of damming rivers, and the propagation of the correct Leninist attitudes to nature, *Pravda* has also carried a number of other news items slanted towards conservation interests.

Thus, on November 20, the "temporary rules" for the conservation of Lake Baikal were announced. These rules, confirmed by the Ministry of Land Reclamation and Water Economy after a long and careful study are aimed primarily at preserving the water resources of the lake. In addition to the purification measures already introduced for industrial plant (especially paper and timber mills) on the tributary rivers, the new rules envisage the maintenance of the rivers themselves. All submerged timber is to be removed from the river beds, and the rafting of timber down these rivers is to be stopped entirely. The transportation of timber by means of "water-stable floats" is to be extended. All waters of the Baikal basin and of the lake itself are to be protected from physico-chemical and biological pollution of all kinds—and, by means of careful organisation and control of holiday centres, sanatoria and rest-homes, from tourist pollution as well. And, in accordance with the Siberian proverb "While there's

forest, there's Baikal", all forestry operations in the area will be carefully monitored to obviate erosion and drying out of the soil, with, in particular, a total prohibition on all tree-felling on slopes with a gradient exceeding 15°.

Again on the subject of forestry resources, a report from Kiev (*Pravda*, November 21, 1974) notes that in the rice-growing areas in the south of the Ukrainian SSR more than 250,000 tonnes of rice straw go to waste each year. In the very same region, there are two paper and cellulose combines which every year consume up to 300,000 cubic metres of timber, brought from the Arkhangel'sk region on the White Sea. If the rice straw, already on the spot, could be used instead of timber, not only would it conserve several thousand hectares of forestland per year, but would also save the cost and logistics of shipping the timber from the extreme north to the extreme south of the USSR.

The "conservation" of human ecology from natural disasters is also well represented: on November 24, a report on measures against erosion by wind and water in the Caucasus, and Transcaucasia, Kazakhstan, the Urals and Eastern Siberia; on November 27, a report from a hydrotechnical research expedition to the Amu-Dar'ya, the "most capricious" of Soviet rivers, where there is a unique problem of bank erosion, locally known as the Deigish, which can lead to extensive flooding, with destruction of hydrotechnical installations, fields and plantations.

And from Vladivostok, in the interests both of conservation and ecology, an interview with Vladimir G. Osipov, Head of the Sector of Oceanic Pelagic Fish of the Pacific Scientific Research Institute of Fisheries and Oceanography, explains the current interest of his Institute in sharks. Not only are they of interest to the ocean ecologist, standing as they do at the apex of the "food pyramid", and to the palaeoichthyologist—they also represent for mankind "a considerable reserve of protein". Shark's liver is rich in vitamins, and the medical industry has been interested for a long time.

Unfortunately, there remains the hazard to life and limb of those deputed to study and catch sharks. Although shark-repellent substances, electrified barriers, or, "most promising", special nets, can be used to protect bathing beaches, it is clearly impossible to study sharks at long range, and "scientists have recorded a large number of tragedies" while working in this field. Like many ecological and conservation problems, shark harvesting seems a matter of theoretical promise but great practical difficulty. □

correspondence

Irrationalism and science

SIR,—It is no longer surprising to find scientists consulting the stars or the I Ching to help make decisions, but it is nonetheless depressing. It is a sign of the times—a pandemic disillusionment with political and social institutions, with the past, present and even the future. In its wake, rationalism hides defensively in a few remaining outposts, while mysticism strides about in its various forms, pointing accusing fingers at the anti-human, anti-God, anti-fun, anti-truth, anti-soul, anti-life of science and rationality. Like all trends, irrationalism is not new, but the speed at which it has come upon us this time around and its proportions are a little frightening.

Uri Geller is a case in point. Ten years ago he would have been a charismatic illusionist; today he is a cult figure, believed by many to have various super-human powers normally reserved for Gods and comic-strip heroes. Scientists join him on television programmes and assure us that his feats cannot be explained by present-day science, neglecting the possibility that they may be best understood in the context of present-day magic. That television, radio and newspaper science journalists should join, or even lead the parade of the occult is perhaps not surprising, but that a prestigious scientific journal like *Nature* should be a credulous participant is a disquieting indication of how far irrationalism has invaded our profession. By acclaiming Geller an important “challenge to scientists” (*Nature*, 246, 321; 1973) and by publishing an inadequately controlled study on Geller’s performance at the Stanford Research Institute (*Nature*, 252, 559; 1974), *Nature* has added its prestige to irrationalism and given it a coveted stamp of scientific approval. Rather than making exceptions and lowering standards in order to publish papers of this kind, surely scientists, and the journals that represent them, have a responsibility to themselves, to science and to society to defend the rational approach against the present wave of obscurantism and anti-reason. Although this will raise cries of “scientific elitism”, it is simply recognising the definition of science, and approach does not demand the disclaiming of unusual phenomena as impossible, but rather an objective assessment of the probabilities in explaining

them, and excluding all natural explanations before turning to supernatural ones. To do otherwise is irrational. A rational perspective on Uri Geller was provided by the *New Scientist* (October 17, 1974).

No matter what the explanations for Geller’s various feats turn out to be, he has served to point up two well-known, but often forgotten facts that deserve publicity and further study. People are remarkably inaccurate observers and reporters of events, even when their professions, such as science or journalism, rely heavily on objective reporting. Suggestion can have powerful effects on the thoughts, sensations and behaviour of most people, which can be useful (as in acupuncture anaesthesia), but which also can be dangerous. It is sobering to think what might happen if anyone believed to have Geller-like powers decided to prognosticate on political or economic issues.

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Pacem in maribus

SIR,—Wendy Barnaby’s otherwise fair account of the *Pacem in Maribus* Convocation (*Nature*, October 11) gives the impression that I said the “non-conventional” living resources of the ocean—such as squids, krill, lantern-fishes—are found mainly in the area beyond the proposed Exclusive Economic Zone. That is not so. The large potential food resources are found both far offshore and rather near coasts. Their geographical distribution is not well known, but the occurrences of high densities are related to zones of high primary productivity. Uncertainty as to jurisdiction over these resources derives less from ignorance of the distributions, than from uncertainty as to the extent of the coastal regime yet to be negotiated. There are many areas of controversy regarding the zonal concept and estimates of the proportion of the total ocean surface which would be under some form of coastal state jurisdiction range from 30 to 90%.

The “unconventional” resource nearest to commercial exploitation is the krill, much of which occurs within 200 miles of the Antarctic continental coastline. With the present Antarctic Treaty in force there would presum-

ably be no EEZ claimed there, but some Parties to the Treaty are considering possible revisions to it to facilitate resource exploitation (*Science*, 184: 776–780; 1974). Of the 12 Parties only two are “developing” states. Seven of the remaining ten are affluent northern hemisphere nations, and three of them have active programmes of research and development on krill.

In future either much of the krill resources will be in the waters of the “international zone” or “high seas” or in the coastal zones of a few countries, including perhaps those which successfully lay claim to the Antarctic resources. Similar arguments can be made for other unconventional resources elsewhere. It may be unwise for the developing countries to assume that the potential living resources of the ocean will lie, as do those resources which are now exploited near to their biological limits, mainly within the jurisdictions of coastal states under an EEZ regime. Further, to which particular types of resources (apart from minerals) such jurisdictions will apply, and under what restraints, remains to be determined, with many difficult questions as yet unresolved.

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Beware the ghost writer

SIR,—One may sympathise with Dr Cater (*Nature*, November 29) who has had his work attributed to Dr Carter but what of the attribution of work to authors who do not exist?

In the journal *Cancer* (29, 1398; 1972) appears a paper by T. Ghose and S. P. Nigam but unfortunately one of the qualifications following Professor Ghose’s name is given as M.R.C.PATH and is printed in the same font as the authors’ names.

This has confused several writers quoting this paper who have referred to it as being by Ghose, Path, and Nigam.

The phantom Dr Path has unfortunately been given bibliographical clothes by the cancer abstracting journal *Excerpta Medica* (Cancer Section) (23; 1973) who give him an entry in their author index.

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news and views

A new stable particle: is charm appearing?

INTENSE interest has been aroused by the observation of a new particle with a mass of $3.1 \text{ GeV}/c^2$. Claims from Brookhaven, New York, where it is called the J meson, and from Stanford, California, where they call it the ψ , agree so far on its major physical properties. The Massachusetts Institute of Technology group led by Sam Ting has seen a very sharp peak in the mass spectrum of e^+e^- pairs, produced when a beryllium target is exposed to the $29 \text{ GeV}/c$ proton beam from the Brookhaven Alternating Gradient Synchrotron. The peak is only about $5 \text{ MeV}/c^2$ wide, which is consistent with the resolution of the apparatus, and its production probability is about 10^{-7} times the probability for producing strongly interacting particles (hadrons). A Stanford-Berkeley group led by B. Richter used the Stanford electron-positron storage rings 'SPEAR' to produce the new particle directly by e^+e^- annihilation. They claim that its width is less than 1.3 MeV , and that it decays to hadrons, to e^+e^- and probably to pairs of muons. In contrast to the low relative probability for producing the particle with protons, the electron-positron interaction probability is enhanced by a large factor as a consequence of ψ (or J) production.

It is clear from the way that the particle is produced that it does not couple strongly to the normal hadrons—the proton and neutron, the hyperons, the pion and kaon, and so on. If it did, then its production rate at Brookhaven should have been much greater. The relatively large production rate at Stanford implies that the ψ (or J) couples to e^+e^- by a simple (lowest order, single photon) electromagnetic process. Weak interactions, as understood at the moment, could not account very easily for the Stanford results.

A particle without strong decays is usually regarded as 'stable', and a width of less than $1.3 \text{ MeV}/c^2$ implies a lifetime of more than 10^{-21} seconds—a long time on the strong interaction scale. If the ψ (or J) carries only known quantum numbers then it is very hard to see why it should be stable. With 3 GeV of decay energy available it is possible to imagine hadron final states with almost any combination of quantum numbers. As long as such final states can exist, then we would expect strong decays of the ψ . Electromagnetic decays do not conserve as many quantum numbers as strong decays do. That is why the well established eta meson can decay electromagnetically into three pions, for instance, even though the conservation laws forbid a strong transition from eta to three pions.

Three of the possible explanations for the ψ deserve mention. The obvious first hypothesis is that it carries a new quantum number which is conserved by strong interactions but is not conserved by electromagnetic processes. This cannot be ruled out, but it is an uneconomical theory since it requires a totally new quantum number to be invented specially to explain this one finding. A second hypothesis is that the ψ is the intermediate boson which carries the weak neutral current (the intermediate vector boson is a heavy particle, probably as heavy as 40 proton masses, which carries the weak interaction; see *Nature*, **250**, 186; 1974, for the present status of experiments on weak neutral currents). But if the boson mass is only

$3 \text{ GeV}/c^2$ we would expect very large differences between the properties of the neutral currents as observed in the CERN Gargamelle experiments with 5 GeV neutrinos and in the Fermi Laboratory experiments with 100 GeV neutrinos. No significant differences have been seen. The third and most elegant hypothesis also incorporates a new quantum number, but one which has already been suggested to explain the absence of strange particle production in neutral-current processes. The ψ may be inhibited from strong decay because of 'charm'; not because the ψ itself is a charmed particle, but because it is made of a charmed quark and a charmed antiquark.

Another well established meson, called the phi, also has electromagnetic decays to e^+e^- . It is not quite a stable particle—it decays by the strong interaction to a kaon plus an antikaon—but it chooses not to decay by the far more accessible channel to three pions. The omega meson, which has all the same normal quantum numbers as the phi, and is lighter than the phi, decays very rapidly to three pions. The absence of a phi to three pion decay has been explained very convincingly by saying that the phi is made from a strange quark and a strange antiquark. In a decay process the strong interaction apparently preserves the quarks that were contained in the decaying particle. The lightest particles which contain strange quarks are the kaons, so the phi either decays to a kaon-antikaon pair, or it decays electromagnetically. If the kaons just happened to be about 10% heavier than they are, then the phi would not be able to decay into them—its mass is $1.018 \text{ GeV}/c^2$ and a kaon has a mass of about $0.49 \text{ GeV}/c^2$.

Perhaps the ψ is a similar object to the phi. Charmed mesons may exist, like the strange mesons we call kaons, but if the lightest of them is more than half the mass of the ψ then the ψ will not be able to decay into a pair of them through the strong interaction. D. J. MILLER

Misunderstandings over C_4 carbon fixation

ECOLOGISTS concerned with energy flow in ecosystems, and particularly those interested in primary energy fixation, await a clarification of the status, both in energetic and ecological terms, of the recently described C_4 pathway of carbon fixation found in certain angiospermous plants. At present the literature presents a rather confused picture. Statements now proliferate which give the impression that there are "two major photosynthetic pathways of carbon assimilation in higher plants" (quoted from Osmond, *Aust. J. Bot.*, **22**, 39; 1974). Although such authors, and perhaps the initiators among their readership, may have a clear idea of what this means, to those unfamiliar with the biochemistry of these photosynthetic processes (which probably includes the majority of ecologists) the implication is that the so-called C_3 and C_4 plants have distinct and dissimilar methods by which carbon is assimilated into the plant tissue. In fact this is not quite true. Although C_4 plants possess a novel system for carbon concentration in

which carbon dioxide initially reacts with phosphoenolpyruvate to form oxaloacetate (a four-carbon acid), a second fixation process is necessary prior to the assimilation of the carbon into plant tissues and is achieved by a release of CO_2 from four-carbon acids within the bundle sheath cells, where it is refixed in the chloroplasts by the conventional C_3 mechanism. This involves ribulose diphosphate (five-carbon) and the production of three-carbon molecules (Hatch, in *Photosynthesis and Photorespiration*, edit. by Hatch, Osmond and Slack, Wiley, New York; 1971; and Black, *A. Rev. Plant Physiol.*, **24**, 253; 1973). A major significance of the C_4 process is its capacity for operating at very low ambient CO_2 concentrations, when it serves to concentrate CO_2 in the mesophyll cells before passing it on to the bundle sheath cells.

The confinement of the Calvin cycle to the chloroplasts of the bundle sheath cells is by no means proven. Evidence for this is mainly negative in that ribulose diphosphate carboxylase activity has not yet been demonstrated in the chloroplasts of the mesophyll cells, whereas phosphoenolpyruvate carboxylase has been shown to be active in the mesophyll cytoplasm. The precise location of the Calvin cycle is still in dispute and some people are inclined to believe that it may occur in the mesophyll (see Coombes, *Commentaries in Plant Science*, **1**, 1; 1973, in *Current Advances in Plant Science*).

Evidently the initial stage of the C_4 system of carbon concentration is not an alternative to the Calvin cycle (C_3) system, but is an additional mechanism which can be advantageous in certain environmental circumstances. In many respects it is comparable to the crassulacean acid metabolism of certain succulents. (Laetsch, *Am. J. Bot.*, **55**, 875; 1968; also see *Nature*, **251**, 380; 1974).

Another area of misunderstanding is the higher photosynthetic rate and, in some cases, higher primary production rate associated with the C_4 mechanism (Zelitch, *Photosynthesis, Photorespiration and Plant Productivity*, Academic Press, London and New York; 1971). Many C_4 species, such as maize and sugar cane, are among the most productive in the world, and this has led to the assumption that the adaptive significance of the C_4 mechanism is one of enhanced productivity. Gifford (*Aust. J. Plant Physiol.*, **1**, 107; 1974) has recently examined this concept by considering the relative advantages of the C_4 system from the molecular to crop growth rate levels of organisation. At the level of initial CO_2 reaction, the C_4 mechanism may have a 60–70-fold advantage (but this is questionable in the light of Bahr and Jensen's work: *Plant Physiol.*, **53**, 39; 1974) over the C_3 system, due mainly to the much lower CO_2 concentrations at which it is able to operate. Consideration of leaf mesophyll *in vivo* shows that this advantage may have fallen to about five-fold at the tissue level due in part, perhaps, to the necessity for refixation in C_4 plants.

A comparison of daytime net photosynthesis of C_3 (for example ryegrass) and C_4 (for example maize) crops with similar leaf area indices and under the same growth conditions shows that the C_4 species may have a 1.5-fold advantage at high light intensities, but this declines with lower light availability. If crop growth rates are measured rather than gas exchange under idealised conditions then one finds considerable variation among both C_3 and C_4 species but there is no evidence for consistently higher growth rates among C_4 plants. Gifford concludes that some of the very high forage yields reported from some tropical C_4 grasses, such as *Pennisetum*, may be related to the greater length of the tropical growing season rather than the higher short-term production efficiency of these species.

Evidently there has been some oversimplification regarding the potential advantages of the C_4 system; high efficiency at the metabolic level is not necessarily maintained up to the level of plant growth rate. A more likely adaptive significance of the C_4 mechanism of carbon concentration

is the maintenance of growth in water-deficient conditions when open stomata and conventional gaseous exchange may result in water stress. In this context Laetsch (*A. Rev. Plant Physiol.*, **25**, 27; 1974) has pointed out that the C_4 mechanism is commonest in plants subjected to intermittent aridity whereas the somewhat related process, crassulacean acid metabolism, is associated mainly with plants experiencing permanently dry conditions. As far as productivity is concerned the ecologist and crop physiologist must evidently apply caution before extrapolating from biochemical data.

PETER D. MOORE

Inertial frames in relativity

THE results of Bishop and Landsberg (page 459) have clarified the rather tangled skein of exposition of relativity theory. They have relevance not only for discussions of the principle of equivalence, which is their concern, but also for the exposition of special relativity which Dingle has attacked on so many occasions. Dingle's attacks have shown the exposition of special relativity to be inadequate, though it is doubtful whether he has shown any deficiencies in the theory.

The key to the whole matter lies in the notion of an inertial frame. Such a frame of reference for space and time measurement occurs both in Newtonian mechanics and in special relativity, and it is assumed in most treatments that the occurrence is the same in each case. Such a view underlies Einstein's heuristic argument by which he managed, as long ago as 1911, to derive from the Doppler shift the gravitational red shift of spectral lines. The essential step here is to transform to a freely falling frame of reference so that the acceleration due to gravity is abolished, by the principle of equivalence, of bodies at a point having the same acceleration. The acceleration in the original frame produces a velocity in the new one, and consequently the Doppler shift. The conclusion is that clocks which were originally synchronised are slower deep in a gravitational field so that the geometry of space-time is modified. Though this argument is only heuristic, it was important for suggesting the principles of general relativity to Einstein, but, as Bishop and Landsberg point out, it is obvious nonsense, since it is entirely carried out in Newtonian physics and therefore a transformation of time is evidently in contradiction to the absolute time concept.

The difficulty lies in the fact that both frames of reference cannot be inertial. Bishop and Landsberg claim to have a theory that gives time stretching in Newtonian mechanics, and gives the Einstein result to the first order in special relativity. Physically, this is not inconsistent with Price's result (*Am. J. Phys.*, **42**, 336) earlier this year with the Einstein formula holding exactly in special relativity. The discrepancy arises only from slightly different definitions of acceleration.

The letter from Bishop and Landsberg, however, leaves unconsidered just what is the intrinsic difference between the two theories, and this seems to lie in the different concepts of inertial frames. In special relativity, such a frame is one in which Newton's laws and Maxwell's equations have their usual form, and it is then a text-book exercise to show that any two such frames are connected by the Lorentz transformation, and that the frames are in uniform relative motion. Essential for this derivation is that we have an inertial frame to start with, so in special relativity there is a six-fold infinity of preferred reference frames. The situation in Newtonian mechanics is completely different: the inertial frame is defined very simply by the form of Newton's laws. Then every frame in uniform

relative motion is also inertial and the transformation between them is then a Newtonian approximation to a Lorentz transformation. But there is no preferred set of frames in the absolute sense of special relativity to start with, because, once gravitational fields are allowed, there is no precise set of forces and any frame of reference can be considered inertial so long as we are prepared to introduce sufficiently strange gravitational fields. The only sense in which the six-fold infinity of frames can be said to be preferred is that of convenience.

The basis of Dingle's long-standing arguments with the relativists seems to be his insistence on the arbitrary nature of inertial frames. Neither his antagonists nor he himself have noticed that this arbitrariness is explicitly denied. The current discussion makes all this a little clearer. C. W. KILMISTER

Element synthesis and isotope anomalies in the early Solar System

from Grenville Turner

THE chemical elements which make up the Solar System were synthesised by various nuclear processes which occur at different stages in the life cycle of stars. Much of the experimental evidence which verifies in detail the operation of these processes is to be found in elemental and isotopic abundances within the Earth, meteorites and recently, the Moon. Many types of process are known to occur and there are different stellar environments appropriate to each; yet significantly, isotopic abundances are, with very few exceptions, within error uniform throughout those parts of the Solar System so far sampled. This observation implies that the material which came together to form the Solar System was at a very early stage homogenised to a very high degree.

Most of the exceptions to this rule (referred to as isotope anomalies) can be understood in terms of the radioactive decay of some long lived precursor isotope and provide the means of determining such things as the age of the Solar System and the duration of some of the element-building processes. Other exceptions in meteorites and lunar samples can be understood in terms of currently operating nuclear processes induced by cosmic rays. These processes are well understood.

Recently two isotope anomalies have been discovered in the very primitive Allende meteorite. Neither anomaly is currently understood but they may

result from nuclear processes operating immediately prior to or during Solar System formation. The first of these anomalies to be found (Clayton *et al.*, *Science*, **182**, 485; 1973) occurs in oxygen in Al-rich inclusions present in Allende. Correlated variations in ($^{18}\text{O}/^{16}\text{O}$) and ($^{17}\text{O}/^{16}\text{O}$) ratios can be most easily understood in terms of the presence of variable amounts of the single isotope ^{16}O . Whether this is the correct interpretation or not it seems difficult to escape the conclusion that one is seeing in Allende the results of the incomplete mixing of the products of nuclear synthesis.

The second recent observation concerns the isotope ^{26}Mg . ^{26}Mg is the daughter product of ^{26}Al , an isotope which has aroused much speculation as a possible heat source during the early history of the Solar System. The need for a heat source capable of heating planetary objects on a rapid time scale arises from a number of arguments. Chemical arguments based on the presence in meteorites and the Earth of volatile elements suggest that solid material originally condensed at relatively low temperatures to form the Solar System. In contrast there is much evidence that a number of processes requiring high temperatures occurred within the first hundred million years of Solar System history; for example, the melting of iron to form iron meteorites, and magmatic differentiation of rocks on the Moon and in certain classes of stone meteorites. The heat source responsible is currently a matter for speculation, ^{26}Al being only one of a number of possibilities.

Stimulated partly by the search for a heat source several attempts have been made to search for the ^{26}Mg anomalies which would result from *in situ* decay of ^{26}Al . Two factors make the search a difficult one. First of all ^{26}Al has a geologically short half life, 7.4×10^5 yr, and so the search must be confined to the most primitive of meteorites to have any chance of succeeding. Second, Mg is itself an abundant element and the anticipated anomalies would be undetectable in all minerals but those most poor in Mg and rich in Al. The search for ^{26}Mg anomalies has had a chequered career and an early claim to have detected them (Clark *et al.*, *J. geophys. Res.*, **75**, 448; 1970) was later shown by an exhaustive series of measurements to have been incorrect (Schramm *et al.*, *Earth. planet. Sci. Lett.*, **10**, 44; 1970).

A recent issue of *Nature* contains one of the latest additions to the saga. Gray and Compston (*Nature*, **251**, 495; 1974) have continued the search in the Al-rich inclusions of Allende, following the observation of the ^{18}O anomalies and encouraged by Sr isotope data

which indicate that these inclusions are the most primitive material known to man (Gray *et al.*, *Icarus*, **20**, 213; 1973). After a series of measurements, on the meteorite and three inclusions, they are able to report a small but significant anomaly for one of the inclusions which would correspond to an 0.4% excess of ^{26}Mg . The (Al/Mg) ratios of the other two inclusions are less favourable and an anomaly, if present at an equivalent level, would be barely detectable. Gray and Compston conclude that if the anomaly is the result of *in situ* ^{26}Al decay then the implied initial concentration of ^{26}Al would represent a significant early Solar System heat source.

A second group of research workers have been searching for Mg anomalies in Allende and their results have also been published recently (Lee and Papanastassiou, *Geophys. Res. Lett.*, **1**, 227; 1974). This work, coming from the laboratory which painstakingly laid to rest the early claims of ^{26}Mg anomalies, indicates that, in Allende at least, anomalies are present. A most significant finding is however that in one of the samples the anomaly is negative—which appears to rule out *in situ* decay of ^{26}Al as the cause of the anomalies.

Lee and Papanastassiou have performed 39 analyses on 21 distinct samples from Allende and found anomalies in nine of the samples. The anomalies are found in the Al-rich inclusions and expressed in terms of the ($^{26}\text{Mg}/^{24}\text{Mg}$) ratio range from -0.17% to $+0.30\%$ with typical uncertainties of around 0.02%. The shifts are much smaller than those found in oxygen, which range up to 5%. Moreover there seems to be no direct correlation with the oxygen anomalies in that the samples with large oxygen anomalies show no magnesium anomaly. There is also no correlation with (Al/Mg) ratio and this in addition to the negative ^{26}Mg anomaly referred to above argues against *in situ* decay of ^{26}Al . The authors suggest a number of possible nucleosynthetic processes which could lead to enrichment or depletion of various Mg isotopes but argue that the absence of a correlation with the oxygen anomalies hinders the identification of a single process as the cause.

In summary there is now a growing body of evidence for the presence in meteorites of the products of nucleosynthesis which have not been thoroughly mixed. To the O and Mg anomalies should be added two as yet unexplained anomalies in the inert gases Xe and Ne (Black, *Geochim. cosmochim. Acta.*, **36**, 377; 1972). The explanation of these effects is a challenge which, if met, will add greatly to our understanding both of the processes of nucleosynthesis and of the early evolution of the Solar System.

A promotory effect of inflammation on tumour initiation

from A. J. S. Davies

To its devotees tumour immunology is an absorbing pastime. To them the facts that T lymphocytes and B lymphocytes and macrophages can all in their different ways be cytotoxic, at least *in vitro*, are mutually compatible. The existence of anti-tumour antibodies that can be thought of as cytotoxic or enhancing or blocking is regarded with joy. The possibility that antibody-tumour-antigen complexes or tumour antigen alone can reduce the aggressive anti-tumour effects of some of the various kinds of hostile lymphocytes becomes part of the grand design. On this euphoric wave of complexity the tumour immunologists are carried as they invent even more and bizarre ways of immunotherapy. Their methodology comes increasingly to resemble some of the early attempts to control bacterial diseases by methods that retrospectively were seen to be non-specific and unreliable.

This is not to say that the tumour immunologists are wrong all the way along the line. There may really be an immunological surveillance mechanism which eradicates most tumours before they become a nuisance. When this mechanism has failed there may be tumour-specific immune responses in all instances which can be manipulated for the benefit of the patient. But the greater the economic pressure on scientists engaged in this biomedical research the more positive will be the assertions, not necessarily justified, that a particular theory is correct. A recent paper in the *British Journal of Cancer* by van den Brenk *et al.* (30, 246-260; 1974) blows a breath of fresh air into the arena.

Van den Brenk and his colleagues have worked on the effects of various inflammatory and anti-inflammatory agents on the survival and growth of clones of injected foreign tumour cells in rats. For example, they found that injection of cellulose sulphate ten minutes before intravenous injection of the Walker-256 adenocarcinoma caused substantial increases in the number of tumour cells starting to grow in the lungs and as a consequence a marked increase in tumour weight. This was even true in immune mice though immunisation always had some protective effect. From other experiments cellulose sulphate is known to be a powerful inducer of inflammation by some mechanism that is not altogether clear. Cellulose sulphate is also an anti-coagulant and can be fibrinolytic.

In spite of the complexity of its effects the authors of the experiments feel and present additional evidence that the inflammatory properties of cellulose sulphate are those responsible for its augmentary effect on tumour seeding and thus tumour growth. In other experiments injection of a lung homogenate is shown to have a similar augmentary effect by means which are thought to be pharmacologically the same as those involved in the effect of cellulose sulphate. Direct effects of either lung homogenate or cellulose sulphate on tumour growth seem to be ruled out in control experiments. Experiments were performed in which tumour and inflammatory agents were injected in the rats' forepaws. Again cellulose sulphate caused some enhancement of tumour growth.

It would be nice to see these experiments repeated with syngeneic tumour implants. But there are many interesting corollaries of the present findings which should be read about in the original. One of the specific points made by the authors is worth underlining here. They say that a situation can arise wherein the survival and growth of tumour cells are inhibited by immunity but stimulated by inflammation resulting from the destruction of participating host and tumour cells. This provides food for thought for tumour immunotherapist and radio-therapist alike especially as Stjernswärd now claims to have evidence (*Lancet*, ii, 1285; 1974) for decreased survival related to postoperative radiation in early operable breast cancer. Van den Brenk and his associates have shown moreover that injection of an anti-inflammatory steroid could reduce the augmentary effect of cellulose sulphate on the seeding of tumour cells. The old fashioned view that tumours should be thought of in the light of the nature-nurture paradigm is perhaps worth reconsideration particularly if damaged cells can nurture (albeit indirectly) their intact tissue-mates.

Superfluid ^3He at zero pressure

from P. V. E. McClintock

LIQUID ^3He has been cooled to temperatures below 0.7 mK in a nuclear demagnetisation cryostat and, under its own saturated vapour pressure, it was found to undergo a superfluid transition at 0.93 mK. The experiment, which was performed at the Helsinki University of Technology by Ahonen, Haikala, Krusius and Lounasmaa, is described in a recent issue of *Physical Review Letters* (33, 628; 1974).

Since the discovery in 1972 that

liquid ^3He under a pressure of 24 atmospheres undergoes two separate transitions at temperatures of about 2.7 and 2.2 K, intensive efforts, both theoretical and experimental, have been made towards gaining an understanding of the two new phases of the liquid. The experiments have, until now, all employed one of two cooling methods: either compressional solidification of ^3He , known as Pomeranchuk cooling, which restricts experiments to the solidification pressure of 34 atmospheres; or adiabatic demagnetisation of cerium magnesium nitrate (CMN), which can be carried out at any pressure. Using these techniques, a remarkable amount of information concerning the nature of the new phases has been gained from a variety of experiments including, in particular, nuclear magnetic resonance (NMR) measurements. It has been shown that both the higher temperature so-called A phase and the lower temperature B phase exhibit superfluid properties; and that, as the pressure is reduced with zero applied magnetic field, the higher temperature transition moves to lower temperatures, while the lower temperature one moves to higher temperatures, with the two transitions meeting in a tricritical point at about 20 atmospheres. Below 20 atmospheres only the B phase is stable, and the transition was found to move to lower temperatures with falling pressure. It was not feasible by demagnetising CMN to follow the transition below about 15 atmospheres and 2.2 mK, so that it was impossible to say whether or not the transition would ever reach zero pressure: it seemed quite credible that ^3He might not be superfluid under its saturated vapour pressure, but would always require the application of external pressure to undergo the superfluid transition.

The ultimate low temperature attainable with CMN is related to the spontaneous ordering of the electron spins on the cerium ions as a result of their mutual magnetic interaction. To reach temperatures significantly below 2 mK an assembly whose magnetic elements interact less strongly is therefore required, and the Helsinki group chose to demagnetise copper nuclei in order to achieve their cooling effect. In essence, the technique is simple. When a large magnetic field is applied at a starting temperature of less than 20 mK, the copper nuclei tend to align themselves, and heat is liberated and is removed by means of a dilution refrigerator. The thermal link to the refrigerator is then broken and the magnetic field very slowly reduced to zero, resulting in cooling as the nuclei disorientate themselves again.

In practice, great care has to be taken in minimising heating due to the

eddy currents which are liable to be induced in the copper as the field is run down; and possible heat leaks, whether through conduction, radiation or mechanical vibration, have to be foreseen and eliminated by suitable design as far as is humanly possible. To avoid the eddy current heating the Helsinki group used a very large bundle of separate high purity 0.1 mm diameter copper wires, insulated from each other along most of their length. The top ends of the wires, well away from the demagnetising field, were all welded together into a piece of copper, which in turn was welded to the copper chamber which held the ^3He .

Earlier calculations had suggested that extreme difficulties might be encountered in achieving good thermal contact between the copper and the liquid ^3He , with a thermal boundary resistance varying as T^{-3} and the probability that nuclear demagnetisation could not be used to cool ^3He below 2 mK. For this reason the cell was designed in such a way as to present as large as possible a surface area of copper to the liquid: most of the volume was filled with a sponge of sintered copper powder, so that the 6 cm³ of ^3He was in contact with about 30 m² of copper surface. In fact, the authors found that the problem was much less severe than had been anticipated, probably owing to thermal transfer through an unforeseen direct interaction between the nuclear magnetic moments of the ^3He atoms and the electronic magnetic moments of manganese impurities in the copper, resulting in a boundary resistance which varied only as T^{-1} .

Demagnetising from a starting temperature of 17 mK over a period of 3.5 h, a final temperature for the ^3He of just below 0.7 mK was achieved. A measure of the quality of the experimental design is given by the fact that it then took 20 h to warm up to 1.0 mK.

By performing experiments at various pressures, and detecting the phase transitions by means of characteristic changes in the NMR signals, the authors traced out the phase diagram, and were able to follow the superfluid transition right down to zero applied pressure at a corresponding temperature of 0.93 mK. This means that, at least in principle, it should now be possible to do experiments involving the free surface of the liquid.

This impressive cryogenic achievement by the Helsinki group means that, for the moment, they enjoy a clear lead over all competitors. It will be interesting to see how they will exploit their advantage, and what illuminating experiments they will perform to eluci-

date the nature of the new superfluids, particularly that of the relatively unknown B phase, now that they have developed the necessary technology.

Receptors for neurotransmitters

from Key Dismukes

THE mechanisms by which a neurotransmitter, interacting with post-synaptic receptors, induces the characteristic neural response remain shrouded in mystery. In recent years much has been learned about hormone receptors in various tissues by probing their interactions with radiolabelled ligands, compounds known to be bound by the receptor with great affinity and selectivity. Synaptic receptors in the brain present a far more difficult problem because of the heterogeneity of neurones, relatively low concentrations of receptors, and their occlusion within the synapse. Nevertheless, there is great impetus to study these receptors, both to elucidate basic neural mechanisms and because many important psychoactive drugs operate through these sites.

The crucial step in biochemically characterising a receptor is to demonstrate that a ligand (often an analogue of the endogenous neurotransmitter) incubated with tissue homogenate selectively binds the receptor in question. Early reports of isolation of acetylcholine receptors from brain have been discounted because the binding was apparently nonspecific, failing to parallel known pharmacological properties of the receptor. Extremely low concentrations of ligand must be used because the limited number of true receptors is soon completely occupied, whereas nonspecific tissue binding increases indefinitely with ligand concentration.

Pharmacologists have described two kinds of acetylcholine receptor: nicotinic and muscarinic. The nicotinic receptor of the electric organ of electric eels was the first to be identified biochemically, and has been investigated extensively by several groups. Although the nicotinic receptor in mammalian brain tissue has resisted definitive biochemical demonstration, several groups have successfully studied muscarinic receptor properties in mammalian brain and peripheral tissues. As long ago as 1965, Paton and Rang detected binding of radioactive atropine, the classic muscarinic antagonist, to the guinea pig intestine (*Proc. R. Soc., B163*, 1; 1965). But the low specific radioactivity of the atropine then available precluded extensive biochemical characterisation.

Recently, Yamamura and Snyder

have examined the binding of a potent muscarinic antagonist, 3-quinuclidinyl benzilate (QNB), using techniques developed for studying the opiate receptor (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 1725; 1974). Binding of ^3H -QNB was found to be almost completely blocked by excess cold QNB, indicating that the interaction was saturable, and thus specific. Muscarinic antagonists and agonists displaced specific ^3H -QNB binding, but nicotinic and non-cholinergic agents showed no affinity for QNB binding sites. These experiments indicated a concentration of muscarinic binding sites similar to that of the nicotinic receptor of the electric organ of the electric eel. The electric organ has been presumed to be extremely densely innervated, but apparently is no more so than the brain is with muscarinic receptors—which is notable, considering that only a small portion of the brain's synapses are likely to be muscarinic.

Burgen, Hiley, and Young have found closely similar features of muscarinic cholinergic receptor binding in rat brain by using ^3H -propylbenzilycholine, an antagonist related to nitrogen mustard which binds irreversibly to the receptor (*Br. J. Pharmac.*, **51**, 279; 1974). Similarly, Soudijn, Wijngaarder, and Ariens have had success with an atropine-like agent, dextimide (*Eur. J. Pharmac.*, **24**, 43; 1973).

Rigorous evidence that QNB binds specifically to acetylcholine receptors was obtained by comparing the pharmacological potency of a number of cholinergic agents with their ability to inhibit ^3H -QNB binding in guinea pig ileum. Acetylcholine is known to produce contractions of the guinea pig intestine through a muscarinic receptor. For each muscarinic agent a close parallel was found between the concentration necessary to block ^3H -QNB binding 50% and the concentration producing half-maximal response of the intestinal strip. Nicotinic agents and non-cholinergic drugs which do not stimulate the ileum did not alter ^3H -QNB binding.

The team of Yamamura, Kuhar and Snyder is now using ^3H -QNB binding to examine the distribution of muscarinic receptors in brain. Binding was examined after destruction of septal cholinergic tracts to the hippocampus (*Brain Res.*, **78**, 320; 1974). Although cholinesterase activity was drastically reduced by this treatment, there was no alteration of ^3H -QNB binding, suggesting exclusively post-synaptic localisation of these receptors. A detailed regional map of receptor concentration has been obtained in monkey brain (*Brain Res.*, **66**, 541; 1974). Because of its extremely high affinity, ^3H -QNB can selectively label the brain's muscarinic

receptors even when administered *in vivo*. After *in vivo* labelling of receptors, it was found possible to perfuse the brain to preserve microscopic structural integrity, thus permitting direct visualisation of receptors by autoradiography (*Brain Res.*, **80**, 170; 1974).

Using procedures similar to those of the acetylcholine work, Young and Snyder have studied the binding of ^3H -strychnine to synaptic membrane fractions from spinal chord and brain stem (*Proc. natn Acad. Sci. U.S.A.*, **70**, 2832; 1973). Strychnine is known to be a potent and specific antagonist of glycine, a major inhibitory neurotransmitter. The binding of strychnine seems to be associated with the synaptic glycine receptor, since the ability of glycine and various other amino acids to inhibit ^3H -strychnine binding closely parallels their glycine-like neurophysiological activity. Furthermore, the distribution of ^3H -strychnine binding in the brain closely resembles the distribution of endogenous glycine, neuronal sensitivity to and nerve-ending uptake of glycine.

Interestingly, Young and Snyder find that glycine and strychnine seem to bind to interacting, but separate portions of the receptor (*Molec. Pharmac.* **10**, 790; 1974). Glycine displaces ^3H -strychnine in a cooperative manner, and its binding can be blocked by various reagents which do not interfere with the binding of strychnine. The inhibitory post-synaptic potential (IPSP) caused by glycine can be reversed by iontophoretic injection of various anions, apparently on the basis of their ability to traverse the membrane's ionic gate for chloride. A close correlation was discovered between the ability of a series of anions to reverse the IPSP and their displacement of bound ^3H -strychnine. This suggests that the binding of strychnine is closely associated with the chloride conductance gate triggered by the glycine receptor. This discovery may be a valuable tool to elucidate the mechanisms by which receptor activation alters membrane conductance.

One of the most intriguing questions of receptor theory is the difference between agonists and antagonists. Why does one type of agent binding the receptor stimulate the characteristic cellular response and another type, binding equally well, produce no effect? Several theories have been proposed, but pharmacological data are not sufficient to distinguish any one. These biochemical binding techniques, being fervidly developed in several laboratories, allow scrutiny of receptor interactions at a level which may answer this and other basic pharmacological questions.

Gallium nitride, a valuable semiconductor

from A. G. Holmes-Siedle

SEMICONDUCTING compounds of elements in groups III and V of the periodic table, such as gallium arsenide (GaAs) could be called the second generation of useful semiconductors. They are isoelectronic with silicon but generally adopt the zinc-blende rather than the diamond crystal form. This leads to a considerable difference in the band structure and to the many-valley feature which permits the very useful Gunn oscillations in GaAs. The array of possible defect centres is intrinsically more diverse than that for silicon because either of the two types of atom present can be replaced in the lattice. This has not prevented the development of devices which employ the radiation emanating from the defect centres during electron-hole recombination. (These devices, light-emitting diodes and injection lasers, have greatly improved numerical displays and may yet revolutionise communications.) By mixing the compounds of gallium, aluminium, arsenic and phosphorous, layered junction structures can be made which emit light of wavelengths varying from $0.5\ \mu\text{m}$ (2.4 eV, green) to $0.9\ \mu\text{m}$ (1.4 eV, infra-red). One member of the series, however, gallium nitride (GaN) is not so easily prepared and does not mix successfully with the other members; this is a pity since it is a material with great potential.

Of all the III-V compounds, GaN has the widest forbidden band gap (about 3.5 eV) and can be induced to luminesce in the blue, violet or ultraviolet regions (the ultraviolet starts at $0.4\ \mu\text{m}$). Also, since the band-edge absorption occurs in the ultraviolet ($0.35\ \mu\text{m}$), pure crystals of GaN are colourless and transparent. In spite of the wide band gap, crystals as grown may often have resistivities as low as silicon and also have fairly high carrier mobilities; if it could be made, the intrinsic or "i" material would be of very high resistivity indeed.

Clearly this is an unusual and potentially useful semiconductor although preparative problems have prevented its use in electronic devices up to now. Not only is it difficult to control the impurities which give high electron concentrations (n-type material); it is equally difficult to find and introduce impurities which produce p-type material. Thus, no good method of forming a p-n junction structure (an integral part of the conventional light emitter) has been found. But there are signs that other practical methods may be found to produce a luminescing

film, for example the generation of avalanche currents by pulsed fields (Pankove and Norris, *RCA Rev.*, **33**, 377; 1972) and there are now signs that GaN may be nearly ready for use in device technology. It may thus be worth while for physicists and chemists to survey some of the known facts about the physics of GaN, aided by a concise review by F. P. Kesamanly (*Soviet Physics, Semiconductors*, **8**, 147; 1947).

A serious drawback to incorporating GaN into the conventional layered III-V alloy laser structures is that, in contrast to all the other III-V compounds shown in Table 1, which crystallise in the zinc-blende form, GaN has the wurtzite structure. GaN was first synthesised in 1932, well before the discovery of semiconducting properties in the III-V compounds. Growth of large single crystals has, however, always proved difficult. Only recently has it been found possible to grow the desired large-area films by epitaxial growth on sapphire, using the disproportionation of gallium chloride and ammonia. This can yield films up to $240\ \mu\text{m}$ thick with an electron density of $2.10^{17}\ \text{cm}^{-3}$ and an electron mobility of $380\ \text{cm}^2\ \text{V}^{-1}\ \text{s}^{-1}$ at room temperature. It has not generally been possible to grow films of lower electron density. But there are reports that Pankove has produced layered structures in which semi-insulating GaN film (that is, electron density presumably well below $10^{14}\ \text{cm}^{-3}$) is overlaid on a strongly n-type film deposited on sapphire. This may give the essential parts of an avalanche electroluminescent diode which can possibly emit light at fairly high power.

While the semiconductor world was awaiting the development of a suitable structure for carrier generation in GaN, investigators were assessing the luminescent transitions by photoexcitation techniques, starting with Grimmeiss and co-workers in 1960 (*Z. Naturforsch.*, **15a**, 799). It was encouraging to find several strong luminescence peaks in the ultraviolet, at 3.47 eV ($0.36\ \mu\text{m}$), 3.29 ($0.38\ \mu\text{m}$) and 3.16 ($0.39\ \mu\text{m}$). R. Dingle and colleagues (*J. Appl. Phys.*, **43**, 3797; 1972) proposed that the 3.4 eV band was the result of recombination of excitons bound to neutral donors and neutral acceptors. Grimmeiss

Table 1 Comparison of properties of III-V semiconductors (300 K)

	GaN	GaP	GaAs	GaSb	
Band gap	3.5	2.24	1.43	0.67	—
Electron mobility	380	110	8,500	400	—
	InN	InP	InAs	InSb	AlSb
Band gap	<1.9	1.29	0.33	0.16	1.63
Electron mobility	—	4,600	33,000	78,000	200

instead attributed the 3.4 eV peak to recombination of free excitons. The doping of GaN with impurities either gives rise to new bands or enhances the triplet. Grimmeiss reports that the doping of gallium nitride with lithium, sodium, copper, silver, gold, zinc, cadmium, barium, mercury, tellurium, tin and lead gives rise to photoluminescence bands with maxima at twelve different wavelengths in the violet to yellow range; mercury produces a band with a maximum at 2.1 eV (0.59 μm , orange). Doping with beryllium, magnesium, calcium, aluminum, and indium produces no new bands but enhances the ultraviolet emission. Soviet workers have found acceptor levels on doping with zinc, cadmium, and lithium; these levels are located 0.40, 0.69, and 0.95 eV above the top of the valence band.

Gallium nitride has been used in the construction of luminescent MIS (metal-insulator-semiconductor) diodes with a Si_3N_4 insulator film 0.1 μm thick (Pankove and Norris, *loc. cit.*). The luminescence spectra of such diodes include a wide band of 2.1 eV energy (0.59 μm , orange) and an ultraviolet band at 3.28 eV. Green-emitting MIS diodes based on zinc-doped GaN and magnesium-doped GaN have been made in this way: the latter material emitted in the violet part of the spectrum. The efficiency of these structures was 10^{-3} . Diodes emitting yellow light and characterised by an efficiency of $4 \cdot 10^{-4}$ were prepared from n-i structures with the high resistivity regions doped with zinc. This is comparable to the efficiency of gallium phosphide green light emitters. In these n-i structures, the light was produced by a pulsed voltage, of, say, 30 V, applied to a metal electrode over the i layer. The light emitted could, of course, be viewed readily through the sapphire substrate, and segmented displays have been made in this fashion. Moreover, it is understood that these devices have operated for several months without degradation in performance. Kesamanly (*loc. cit.*) also reports that GaN will alloy with indium nitride and that the band gap is adjustable thereby.

It thus seems that GaN is on the threshold of becoming a new tool in the hands of the semiconductor physicist. It will be more versatile than other III-V compounds both because the diodes will emit light over the whole of the visible range and because GaN is completely transparent in the visible. It will be of great interest to see what benefits this will bring to our technological repertoire and which country (the United States, United Kingdom, Soviet Union, Germany) leads the way in developing new gadgets—say a white-light laser or solid television display—from this advance.

Dilatancy without fluid flow?

from Peter J. Smith

It should be quite possible to predict earthquakes on a purely phenomenological basis with little, if any, understanding of the physical process involved. All that would be necessary would be to find a premonitory effect which is common to all earthquakes, or at least to a class of earthquakes large enough to give prediction some practical significance. In recent years, for example, it has been observed that, before some shallow events, the seismic wave velocity ratio V_p/V_s suddenly decreases and then rises again to its normal value just before the shock takes place. This phenomenon may or may not turn out to be a way of predicting shallow earthquakes on a routine basis; but whether it does or not need not depend on any insight into the cause of the velocity changes.

Nevertheless, there is some advantage in having a physical model to explain the observations. For one thing, it tends to inspire confidence; and if there is one thing likely to be required in abundance when it comes to practical prediction, it is confidence. More specifically, an understanding of the mechanisms involved may lead to the discovery of new relationships, and thus bring practical prediction closer. For example, there can be little doubt that the dilatancy model, first proposed by Nur (*Bull. Seismol. Soc. Am.*, **62**, 1217; 1972) and subsequently developed and modified by others, has done almost as much as the observations themselves to produce the new wave of optimism in prediction work. By relating the various premonitory effects such as V_p/V_s changes, resistivity variations and the migration of seismicity to a common mechanism and a common precursor time-earthquake magnitude scale, the dilatancy theory has produced the comfortable feeling that everything is beginning to fit nicely together.

But is the theory correct? Or to put the question in the more limited form that Stuart (*Geophys. Res. Lett.*, **1**, 261; 1974) now puts it: are the current dilatancy models involving fluid flow correct? Insofar as there have been no serious objections to the dilatancy-fluid diffusion mechanism, the question may seem superfluous. But as Stuart points out, the theory apparently defies Occam's razor by introducing fluids without a proven need. Moreover, there are good scientific reasons for questioning the occurrence of fluid diffusion, described by Stuart as the "requirements of ubiquitous finite permeability, existence of pore fluid, and ostensibly great distances involved for large

earthquakes".

Stuart's new model has the earthquake occurring within a relatively thin shear zone which has mechanical properties quite different from those of the crustal rocks further away. In the shear zone, the energy applied during deformation is dissipated by creep. In the surrounding crustal rocks, on the other hand, the deformation energy is stored elastically until such time as it is released in an earthquake. Assuming that the two regions are each homogeneous and in welded contact, their stresses at any time will be similar but the strains will differ significantly. To a first approximation the outer rocks will have a linear stress-strain relationship, whereas the shear zone is assumed to have a stress-strain curve possessing a maximum stress.

The crustal rocks in Stuart's model thus have properties comparable to those of the material in conventional dilatancy models; and these rocks are likewise assumed to possess cracks and fractures. The crucial difference is the non-linear behaviour in the thin shear zone. As the stress applied to the combined system increases, dilatancy will occur as the cracks open in the crustal rocks, giving rise to a decrease in V_p/V_s . But as the stress continues to increase, dilatancy in the crustal rocks will reach a maximum and then decrease as the cracks begin to close up again, V_p/V_s will correspondingly increase. The earthquake will occur when V_p/V_s is back to normal, if the energy conditions are favourable. (If the conditions are not so favourable, Stuart's model suggests that rapid creep might occur instead; in other words, the model implies that similar premonitory events herald both earthquakes and accelerated creep.)

It seems, therefore, that the V_p/V_s changes observed in the field may be explained quite well without involving fluid; the inflow of fluid (to raise V_p/V_s) in conventional dilatancy models is replaced in Stuart's model by closing cracks. Fluids may indeed be present, but they are not necessary to the argument. Qualitatively, the two models predict similar precursory variations in V_p/V_s , resistivity, and so on.

But there is at least one difference which should enable the models to be distinguished. In Stuart's model the dilatancy reaches a maximum and decreases to vanishing point before the earthquake as the cracks close. In the diffusion model the dilatancy goes on increasing up to the earthquake. Moreover, because it is diffusion controlled, the dilatancy in the latter case will only decrease slowly after the shock. In time, therefore, it should be possible to test the validity of the two models by geophysical measurement.

review article

Retarded cores, black holes and galaxy formation

John Gribbin*

Recent astronomical observations have given rise to suggestions that black holes may reside at the centres of many or all galaxies. The evidence remains equivocal; and in the meantime there is a theoretical variation on this theme, in which galaxies grow on retarded cores — 'white holes' — which have been present since the initial big bang.

DURING the past few years the concept of black holes has moved out of the specialist astronomical literature into a broader arena of discussion. This has come about chiefly because of the interest aroused by the discovery of sources of intense X-ray emission which seem, from the periodic variations of systems which are taken to be binary stars, to be too massive to exist as stable stars without producing visible optical radiation. Since no optical counterpart can be detected for these objects, it has been suggested that they have evolved from burnt out massive stars which have collapsed, now that nuclear reactions no longer provide the energy necessary to hold them up, into black holes.

The idea is certainly attractive, and it has the advantage that such a collapsed object would indeed be able to 'drive' the observed X-ray emission, through the conversion of gravitational potential energy into radiation as matter falls into the black hole. It is not yet clear, however, that it is strictly necessary to invoke black holes to explain the X-ray sources observed so far; the evidence is persuasive, but other more or less bizarre ideas (such as differentially rotating white dwarf stars, or models based on triple rather than binary systems, see ref. 1) can also be made to fit the observations.

Whether or not they prove to be the correct explanation of X-ray sources, the excitement aroused by recent work has tended to obscure the fact that black holes are not a recent idea. In fact, such objects have been contemplated by the mathematicians for many years, and whether or not stellar mass black holes exist in star systems in our Galaxy that kind of object could play an important part in the evolution of the Universe, or of individual galaxies. In many ways, the concepts involved in studying the consequences of the existence of such large mass black holes are just as exciting as the possibility that smaller black holes exist in our immediate astronomical neighbourhood; so this may be a good time to examine recent progress from these older ideas.

Observational evidence

It seems likely that elliptical galaxies contain massive 'black holes'—objects collapsed within their Schwarzschild radii—in their nuclei (see, for example, Wolfe and Burbidge²). The principal evidence in favour of this concept is the 'missing mass' problem; the average mass of a bright elliptical is $8 \times 10^{11} M_{\odot}$ and the mass-to-light ratio is about 70, implying that the light from these ellipticals can be explained in terms of the emission from stars which make up only some 25% of the mass. There is no similar mass-to-light problem with spirals, but the data are insufficient to rule out the possibility that spirals (including our own Galaxy, see ref. 3), also contain black holes.

But if black holes do exist at the centres of galaxies, it is

something of a problem to account for the evolution of them in terms of gravitational collapse. Wolfe and Burbidge mention in their paper the possibility that such central objects might be related to the 'retarded cores' discussed by Novikov⁴ and by Ne'eman⁵, and by others since 1965. A straightforward calculation in the Newtonian approximation shows that the constraints imposed on the nature of elliptical galaxies by assuming that they form on such cores are in complete accord with the limits imposed on the masses of the central black holes from a study of the structure of elliptical galaxies.

Limits on the size

Following Wolfe and Burbidge², I take the energy generation 'requirement' of an elliptical galaxy to be 10^{61} erg, allowing the galaxy to become a strong radio source. This is equivalent in mass terms to $10^7 M_{\odot}$, and since there is evidence that recurrent outbursts can occur in radio galaxies, a total of some $10^9 M_{\odot}$ seems to be required to power the observed phenomena. For such a mass, the Schwarzschild radius (R_s) is about 10^{-4} pc; for a mass of $10^{11} M_{\odot}$ R_s is about 10^{-2} pc. There seems little doubt that the energy which drives radio sources must be gravitational in origin, and according to Wolfe and Burbidge "on the basis of conventional theory, it must be argued that ellipticals which have given rise to radio sources contain black holes", because of the requirement that large masses must now be concentrated within regions a few light years across in order to explain the energy production.

From studies of the velocity dispersion in the inner parts of some elliptical galaxies Wolfe and Burbidge are able to set limits on the size of any central black hole. I shall assume that the required mass is not present in the form of many smaller black holes, although this possibility cannot be ruled out by present data. It then turns out that the mass required to explain the observed distributions must in general be less than some $10^{10} M_{\odot}$, and that for systems which are relaxed the central mass must be less than $10^9 M_{\odot}$ (ref. 2).

Possible size of retarded cores

The concept of retarded cores of expansion arises naturally if we consider time reversal of the present expanding Universe. To an observer A in a region which is less dense than the region inhabited by another observer B there must come a time when observer B disappears behind the event horizon of a black hole as his region of space contracts within the value of R_s appropriate for the density it has.

By considering the time reversal of this situation, it seems reasonable to argue that in an expanding universe which is not of uniform density observers in regions of less than average density will see regions of greater density expanding away from their Schwarzschild radii. Such relatively dense regions would therefore appear as 'white holes', and their expansion would presumably follow essentially the same rules as the

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expansion of the whole Universe. It is postulated^{4,5} that the expansion of such cores is delayed in some way, hence their description as 'retarded cores'. Eardley⁶ has suggested that because of accretion any white hole existing in the Universe would rapidly be turned into a black hole, because of accretion from its surroundings.

This is more or less what one would expect from common-sense arguments, but the Universe may not be run in a 'commonsense' way. Since the whole concept depends to some extent on tinkering with the boundary conditions of the big bang, it is not unreasonable to argue (P. C. W. Davies, personal communication) that the Universe adjusts itself in some way to counteract the tendency of white holes to extinguish themselves by accretion. That argument is not entirely satisfactory. But the case against white holes is not yet proven, and in what follows the existence of either black or white holes will suffice to 'seed' galaxy formation. Even if an initial white hole or delayed core is converted into a black hole, its mass will still act on outside material in exactly the same way.

It could be argued that there is no evidence that the Universe was ever in a suitable state of inhomogeneity to give rise to such cores; but then, there is no evidence that it was not, and it also seems (see Misner, Thorne and Wheeler⁷) that an initially random 'mixmaster' type of universe would rapidly evolve into a state very like an Einstein-de Sitter universe with some inhomogeneity. What happens to the region of the universe near to such a retarded core as the universe expands? Locally, gas will be restrained from the general expansion by the gravity of the core, and if stars form in the resulting gas cloud we may expect a galaxy to be produced.

There seems no need to look further than the Newtonian analogy of the Einstein-de Sitter universe for a first model to describe the behaviour of the cloud around the retarded core. For a homogeneous cloud of mass M we have

$$r^2 = 2GM/r$$

Ideally, the calculation should be carried through with some mass—the retarded core—kept within some confine (presumably its Schwarzschild radius) at the centre of the cloud from the start of expansion. But that involves a full mathematical treatment, and a simpler calculation in the Newtonian approximation may suffice to indicate the broad outlines of what is going on. The trick is to allow a mass μ to appear at the origin of r at a time when $r = r_0$. As it stands, that assumption can be as accurate as we wish, since we have not yet specified either μ or r_0 . And if such a mass does appear then subsequent expansion will obey the equation

$$r^2 = [2G(M+\mu)/r] - [2G\mu/r_0]$$

so that the cloud expands to a maximum radius given by

$$r_{\max} = (1 + M/\mu)r_0 \sim (M/\mu)r_0 \text{ if } M \gg \mu$$

and then falls back. Since M is the mass interior to r , the outer regions expand to greater maximum radius than the inner regions.

In the Einstein-de Sitter cosmology with spherical symmetry we have

$$ds^2 = d\tau^2 - S^2(\tau)(dR^2 + R^2 d\Omega^2)$$

where

$$S \propto \tau^{2/3} \text{ and } c = 1$$

Transforming to locally flat space-time (for an observer at $R = 0$) gives $ds^2 = dt^2 - dr^2 - r^2 d\Omega^2$ + (local gravitational effects) and the local gravitational effects may be included as a fourth order term such that

$$ds^2 = dt^2 (1 - 2GM/r) - dr^2 - r^2 d\Omega^2 \quad (1)$$

where M is the mass interior to r , proportional to r^3 for uniform density. Equation (1) is the Newtonian result to first order, and the effect of a mass μ at $r = 0$ may be included by writing

$$ds^2 = dt^2 [1 - 2G(M+\mu)/r] - dr^2 - r^2 d\Omega^2$$

where M is now the mass of the cloud interior to r and not the total mass (cloud + core) interior to r .

It is possible to represent the solution of the local gravitational problem by a power series in the dimensionless parameter $2G(M+\mu)/(rc^2)$ which is small for a local problem. The first term in the series is the Newtonian solution for the effect of μ and may be used to good approximation provided that the second term in (GM/rc^2) is smaller than the first order term involving μ , that is, if

$$2G\mu/rc^2 \gg [2GM/rc^2]^2$$

This must hold for all r , including $r = r_0$, so

$$r_0 \gg (M/\mu) 2GM/c^2$$

if the problem is capable of solution by the Newtonian approximation. This corresponds to

$$r_{\max} \gg (M/\mu)^2 2GM/c^2 \quad (2)$$

The argument here comes close to being circular, but seems fairly plausible. What we have found is that if the Newtonian approximation is valid, then the maximum radius reached by the expanding cloud with a retarded core at its centre satisfied inequality (2). Now, it is no secret that the whole purpose of this calculation is to come up with a mathematical description of something which looks like a galaxy. So we can put a typical galactic radius, say 3×10^{22} cm, into inequality (2) as the value of $r_{\max}$. If that is done, the inequality holds with a central mass μ of $10^9 M_\odot$ acting to retard the expansion of a cloud of mass $M = 10^{12} M_\odot$. With those figures, we can also set a limit of 3×10^{19} cm on r_0 .

Putting all these figures together, we can say that for such a mass ($10^9 M_\odot$) the value of r_0 is much greater than the appropriate value of r_s . And, most important of all, we do indeed find that for galaxy-like objects the assumption that $\mu \ll M$ is plausible.

All this does not, of course, prove that there must be collapsed objects at the centres of galaxies. But it suggests that the idea is not unreasonable. That is the best that can be hoped for for many astronomical ideas. The result is, however, suggestive in the light of the limit set on any central mass by Wolfe and Burbidge.

Nature of the resulting galaxy

If we accept this result at face value it is simple to determine the optical appearance of the resulting galaxy. By considering shells of thickness dr at distances r from the centre of the gas cloud, we have a mean density at r given by

$$\rho_r = (\text{constant} \times r^{-2/3} dr) / (r^2 dr) \propto r^{-8/3} \quad (3)$$

simply from dividing the mass of the shell by its volume. If stars form throughout the cloud we may take relation (3) as indicative of the number density of stars at r , and if all stars have the same luminosity function then the emissivity per unit volume at r is simply

$$E_r \propto r^{-8/3}$$

so that the intensity distribution measured by an outside observer will be

$$I_r = r E_r \propto r^{-5/3}$$

This is assuming an initially spherical distribution; but the resulting power law is in striking agreement with observations of elliptical galaxies and rather better than Hubble's well known empirical inverse square law.

Deviations from the spherically symmetric expansion may be considered by imposing a rate of strain tensor and a rotation of the cloud about the central mass. Hoyle and Narlikar⁸ have considered similar problems within the rather different framework of one of the C-field variants on the steady state model. They find that in such a situation the galaxy formed will be ellipsoidal with unequal principal axes, so that we would have something very similar to the elliptical galaxies observed today.

There remains the problem of spiral and irregular galaxies, and I do not wish to postulate any detailed model to account for their formation here (but see ref. 9). Evidence which suggested a two-component structure to our own Galaxy might, however, be taken as a hint that the spiral structure is a later appendage which has grown on to a retarded core type of central galaxy; and since rotation is only a second order effect which ought to be rapidly damped in the kind of expanding cloud envisaged, any evidence that elliptical galaxies are in general rotating would make the model outlined above untenable.

It is, however, striking that Wolfe and Burbidge say that any central black hole must be of mass less than $10^9 M_{\odot}$ to

account for the structure of observed ellipticals, that the retarded cores hypothesis leads to exactly the same limit and that just this mass is required for the energy generation mechanism in radio galaxies. The model removes problems of how the collapsed objects formed, and the notion of such retarded cores as 'white holes' at the centre of galaxies encourages obvious speculations about the processes in QSOs, Seyferts and the like where matter seems to be pouring out into the Universe from local regions of space.

Even if it is necessary to adjust the boundary conditions to accommodate the existence of white rather than black holes at the centres of galaxies, that would be no worse than other ideas which have been put forward and which explain the energy produced in such objects in terms of new laws of physics and even the creation of matter.

¹ *Nature*, **247**, 333 (1974).

² Wolfe, A. M., and Burbidge, G. R., *Astrophys. J.*, **161**, 419 (1970).

³ Lynden-Bell, D., *Nature*, **223**, 690 (1969).

⁴ Novikov, I. D., *Astr. Zh.*, **41**, 1075 (1964); transl. *Soviet Astr.-AJ*, **8**, 857 (1965).

⁵ Ne'eman, Y., *Astrophys. J.*, **141**, 1303 (1965).

⁶ Eardley, D. M., *Phys. Rev. Lett.*, **33**, 442 (1974).

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articles

Towards gamma-ray lasers

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We suggest that two types of γ -ray laser are possible: a gently pumped variety utilising isomer separation and the Mössbauer effect, and a vigorously pumped kind using fast neutron excitation of compressed matter.

In this article we will discuss the operation of γ -ray lasers, namely devices which produce coherent, monochromatic, unidirectional electromagnetic radiation from de-excitation of excited nuclear levels. We will describe the necessary conditions for γ -ray laser operation, and consider some possible means of satisfying them.

The lifetimes for spontaneous γ -ray emission from excited nuclei range from 10^{-17} to 10^{-6} s if ΔJ , the angular momentum change in the transition, is small (≤ 2). For low energy transitions with large angular momentum changes, the lifetime can be much longer. For example, for $\Delta J = 4$ and energy change ~ 100 keV, the radiative lifetime can be several years¹. The existence of long lived excited nuclear states is intriguing from the point of view of achieving γ -ray laser action, because relatively modest pumping powers would be needed to produce population inversions. The opportunity of exploiting the multi-MeV binding energies of nucleons in nuclei to pump long lived

transitions between even high lying nuclear levels with the thermal neutron fluxes and charged particle accelerator beams is also notable. Moreover, the extraordinarily long life of transitions with large ΔJ makes it potentially possible to create nuclear population inversion through atom-by-atom physical segregation of the excited isomeric state from predecessor nuclei and other states of the same nucleus²⁻⁴. To take such advantage of long lived nuclear excited states, however, the spectral width $\Delta\nu$ of the γ -ray emission line must be sufficiently small. The threshold inversion density for producing γ -ray laser action is proportional to $\Delta\nu\tau_{\text{spont}}$, where τ_{spont} is the spontaneous radiative lifetime of the excited state. Thus a large value of τ_{spont} must be compensated by a small value of $\Delta\nu$, in order that the density of the laser medium be that of a solid or less. If sufficiently small values of $\Delta\nu$ cannot be achieved, then shorter lived excited levels, together with a density of the γ -ray laser medium higher than that of a solid, would be required.

Unlike X-ray line widths, γ -ray line widths are substantially unaffected by the properties of the medium, with one notable exception: 'recoil-less' emission in a crystal (the Mössbauer effect)⁵. Indeed, it is just the inability of the medium in which isomeric nuclei are embedded to mix excited nuclear states of high relative multipolarity significantly into ones of lower multipolarity which permits their extraordinary metastability.

Normally, γ -ray line widths are determined by either Doppler broadening (in a high temperature medium) or broadening arising from recoil of the emitting nucleus. The fractional spectral width $\Delta v/v$ for thermal Doppler broadening is

$$(\Delta v/v)_{\text{Dopp}} \leq 10^{-3} (\theta/A)^{1/2} \quad (1)$$

where θ is the temperature of the medium (keV), and A is the mass number of the nuclei of interest. The fractional line width caused by recoil of the emitting nucleus is

$$(\Delta v/v)_{\text{recoil}} = \hbar\omega/2Mc^2 \approx 10^{-5}k \quad (2)$$

where k is the photon energy in MeV and M is the mass of the recoiling nucleus. Except for the special circumstance of recoil-less emission, the recoil broadening sets a lower bound on the γ -ray line width. In the case of recoil-less emission, the emitting nucleus is bound in a crystal whose Einstein oscillator energy is at least comparable to the kinetic energy acquired by an isolated, γ -ray emitting nucleus. The recoil broadening of such a crystal is negligible because substantial parts of it can take up the recoil momentum. The spectral widths in such cases can be much closer to the natural widths; γ -ray line widths $\Delta v \sim 10^5$ Hz have been observed⁶ for transitions with $\tau_{\text{spont}} \sim 10^{-6}$ s by Mössbauer spectroscopic techniques. By growing very pure crystals with minimal line-broadening lattice stresses^{7,8}, much smaller emission line widths should be attainable with long lived nuclear isomers.

These considerations suggest two possible classes of γ -ray laser: (i) those which operate on allowed (E1 or M1) or weakly forbidden (E2 or M2) transitions, necessarily under vigorous pumping conditions, with $10^{-5} \lesssim \Delta v/v \lesssim 10^{-3}$; (ii) those which operate on forbidden transitions (for example, E3 or M3), in an environment such that $\Delta v/v < 10^{-15}$ (refs 7 and 8). In (i), the threshold inversion densities will generally be higher than solid densities, so the vigorous pumping must usually be accompanied by compression of the γ -ray laser medium. In (ii), attainment of the small values of Δv needed require recoil-less emission, so the laser medium must be gently pumped, at a temperature less than the Debye temperature^{2,3}.

As noted previously⁴, it is not necessary to use cavity-bounding mirrors to produce γ -ray laser action. Indeed, the extremely high minimum fluxes and fluences of a pulsed γ -ray laser would destroy such reflectors. The nature of gently pumped devices may allow the use of Bragg diffraction-type^{9,10} cavity mirrors; however, high intrinsic losses (≥ 2 –3 dB per pass) make them only marginally useful. We therefore take axial superfluorescence to be a necessary condition for γ -ray laser operation, and (quite conservatively) require

$$\alpha l \gtrsim 30 \quad (3)$$

where α is the net gain and l the length of the cylindrical, population-inverted medium. Concurrently, in order to avoid radial superfluorescence, the diameter d of the laser medium must be small compared with its length,

$$d \ll l \quad (4)$$

but at least comparable to $(\lambda l)^{1/2}$ and large relative to $(\lambda/a)^{1/2}$, in order to avoid severe diffraction losses. In the absence of losses, equation (3) implies that the net inversion density N^* must satisfy (L.W., G.C., S. Slutz and G. Zimmerman, UCRL75184, 1974).

$$N^* \gtrsim 30 (8\pi/\lambda^2 (\Delta v\tau_{\text{spont}}/l)) \quad (5)$$

where λ is the γ -ray wavelength. Equation (5) is the threshold condition for laser action. We now discuss how this threshold condition might be satisfied for each of the two classes of γ -ray laser.

Vigorously pumped lasers

We consider, as a possible technique for population inversion, the pumping of nuclear excited states by very high neutron fluxes. The γ -ray laser would consist of a DT thread of milligram mass, loaded with perhaps 1 atom-% of the higher Z nuclei on whose neutron-excited, population-inverted metastable levels the laser will depend for its operation. (Lasing might also take place between levels of population-inverted capture, spallation or fission product nuclei.) Recalling that characteristic 14-MeV neutron excitation/break-up cross sections on such nuclei are 10^{-24} cm², we note that time-integrated neutron fluxes in compressed-and-ignited fibres of the order of 10^{24} cm⁻² may be obtained over picosecond time scales by compression to densities of 10^4 g cm⁻³ (comparable to those proposed for a laser controlled thermonuclear reactor¹¹). The corresponding fluxes ($> 10^{35}$ cm⁻² s⁻¹) are more than sufficient to give a high probability of excitation/break-up on time scales characteristic of allowed γ -ray transition of energies < 1 MeV.

The threshold inversion density is $1.2 \times 10^{28} k^3$ cm⁻³, assuming $l = 1$ cm, $\tau_{\text{spont}} = 10^{-12}$ s and taking $\Delta v/v = (\Delta v/v)_{\text{Dopp}} \approx 10^{-3}$, from equation (1), using a typical fusion microexplosion ion temperature of ~ 100 keV and $A \approx 10^2$. We conclude that it is possible to achieve γ -ray laser action with $k \lesssim 0.2$ in a suitable cylindrical fusion microexplosion for which the space-time power programming of the implosion-driving laser pulse streaked the microexplosion along such a fibre at about the speed of light. Laser pulses of about 10^5 J per mm of length would apparently be required to implode and ignite the fusible material which produces the neutron fluxes needed to pump the emitting nuclei.

Of course, attainment of large inversion densities is not necessarily sufficient for the attainment of laser action, because of γ -ray laser losses in the medium. In order that the gain due to stimulated emission of a vigorously pumped γ -ray laser exceeds the loss due to Compton scattering, the dominant opacity mechanism,

$$\tau_{\text{spont}} < 10^{-14}/(\bar{Z}k^3) \text{ s} \quad (6)$$

where $\bar{Z}$ is the average atomic number of the laser medium. This is an extremely stringent condition and indeed, there are no γ -ray transitions with $k < 0.2$ presently known to us which satisfy this condition.

Table 1 Data for a gently pumped γ -ray laser

k (MeV)	λ (Å)	$\lambda^2/8\pi$ (barn)	Δv_{max} (Hz)	Δv_{min} (Hz)	ζ_{min}
0.01	1.24	6×10^8	30.0	1.0	30
0.05	0.248	2.4×10^5	1.0	0.01	100
0.10	0.124	6×10^4	0.3	0.001	300
1.00	0.0124	600	0.003	0.0001	30

The Table shows, as a function of γ -ray wavelength, the approximate maximum allowed line width Δv_{max} for a gently pumped γ -ray laser ($l = 3$ cm, $\tau_{\text{spont}} = 10^{-12}$ s), the internal conversion-limited minimum line width, assuming $\tau_{\text{spont}} = 10^{-12}$ s, $\alpha = 10 (0.1/k)^3$, and the minimum figure of merit $\zeta = \sigma_{\text{max}}/(1 + \alpha) \sigma_{\text{abs}}$ needed to operate at the maximum allowed width.

Gently pumped lasers

We have noted that, to exploit recoil-less emission, the excited nuclei must be incorporated in a crystal lattice which is kept sufficiently cool³. This suggests that the nuclei must be excited before they are assembled into a crystal lattice. As a practical matter, it seems that nuclei may be excited and then embedded in a recoil-less environment (for example, irradiated in a nuclear reactor, separated from other isotopes and nuclei of the same A and Z but different excitation levels, and then crystallised into a thread of suitable composition) only on time scales $> 10^2$ s; so we are compelled to use relatively longlived nuclear isomeric states. Moreover, the strengths of chemical bonds of most substances imply that substantially recoil-less environments exist only for $k \lesssim 0.1$. We are therefore most interested in E3 or M3 transitions. We note that the collective nature of these excited nuclear states implies large isomer shifts of electronic spectral lines¹², comparable to isotope shifts, which greatly facilitates isomer separation by photochemical/photophysical means (ref. 13 and T. Axelrod, A. Bernhardt, T. O'Leary, J. Marling, R. Keeler and L. W., UCRL74904, 1973).

acoustic isolation would also be required to minimise axial velocity gradients in the medium. Indeed, laser action itself will lead to a relatively slight, variable acceleration of the medium, which is minimal if the system emits collectively. Such super-radiant emission^{15,16} is likely, since the 'cooperative length' for collective de-excitation, $l_c = (2\pi\tau_{\text{spont}}/\lambda^2 N^*)^{1/2} \gg l$.

Internal conversion is an important limitation on gently pumped γ -ray lasers because of the high multipolarity and relatively low energy of the transitions of interest. For E3 or M3 transitions, the (dominant) K-shell internal conversion coefficient α_K is of the order of 10 for $k \approx 0.1$. Thus our assumed radiative lifetime τ_{spont} of 10^4 s corresponds to an actual half life $\tau_{1/2} \approx 20$ min, which would be the maximum time allowed for isomeric state separation, followed by crystal formation and conditioning. Since $\tau_{1/2} = (\alpha + 1)\tau_{\text{spont}}$, where $\alpha = \alpha_K + \alpha_L + \dots$ is the total internal conversion coefficient, we also have $\Delta v > (\alpha + 1)/\tau_{\text{spont}}$.

Photoelectric absorption also severely limits the available γ -ray laser parameter space. The photoelectric cross section per atom in cold matter, σ_{photo} , is of the order of Z^4 ($0.01/k$)³ $\times 10^{-26}$ cm². This photoelectric cross section may be quite large

Table 2 Nuclear isomers considered most promising for gently pumped γ -ray laser action

Isomer	k (MeV)	λ (Å)	σ_{max} (barn)	σ_{abs} (barn/atom)	α	ζ	$\tau_{1/2}$ (min)	Class	Production
^{60m} Co	0.059	0.21	1.8×10^5	120	41	37	10.7	M3	⁵⁹ Co, 100%; 18 barn
^{79m} Se	0.096	0.13	5×10^4	84	7	112	3.9	E3	⁷⁸ Se, 24%; 0.4 barn
^{81m} Se	0.103	0.12	5.8×10^4	70	9	92	57	E3	⁸⁰ Se, 49%; 0.1 barn
^{77m} Br	0.108	0.11	5.2×10^4	70	6	124	4.2	E3	⁷⁷ Se, (p, n) ⁷⁷ B
^{99m} Tc	0.143	0.09	3×10^4	70	30	14	350	M4	⁹⁹ Mo, β^- decay

σ_{max} is the approximate maximum stimulated emission cross section possible, σ_{abs} the photoelectric absorption plus Compton scattering cross sections at the transition energy per atom, α the total internal conversion coefficient and the figure of merit of the isomer, $\zeta \equiv \sigma_{\text{max}}/(1 + \sigma)$ σ_{abs} $\tau_{1/2}$ the excited state half life. Also shown are the multipolarity of the radiative decay and the most promising means of production of the isomer, including the fractional natural abundance and thermal neutron capture cross section (in barns) to the isomeric state of its precursor. ^{60m}Co, ^{79m}Se and ^{99m}Tc are considered especially interesting as they are formed substantially population-inverted.

Maximum values of Δv which allow attainment of threshold γ -ray laser inversion densities below those of a solid (5×10^{22} cm⁻³) are shown in Table 1, assuming $\tau_{\text{spont}} = 10^4$ s and $l = 3$ cm. Although required values of Δv are smaller than the smallest so far measured to present⁶ ($\approx 10^6$ Hz), they are much larger than the typical natural widths. It therefore seems reasonable to expect that the required values of Δv may be attained, using carefully prepared crystals (such as metal whiskers which have only a single axial screw dislocation and are effectively perfect) to minimise inhomogeneous line broadening caused by lattice irregularities. Even in such crystals, however, there would be inhomogeneities arising from differences between the upper and lower states of the emitting nuclei. For instance, the magnetic dipole-dipole interaction between neighbouring nuclei can be $\sim 10^3$ Hz and would result in disastrously large line broadening in even a perfect crystal. Fortunately, such interactions will time-average to much smaller values, producing the required line narrowing, if the nuclear spin temperature is not too low. Such nuclear spin temperatures ($\gtrsim 10^{-6}$ K) may be attained by many means, if necessary, such as the application of radiofrequency pulses to the γ -ray laser medium¹⁴. In order to avoid line shifts caused by external magnetic and electric fields, electric field gradients across the laser medium must be kept below 10^7 V cm⁻¹ and ambient magnetic field intensities must be $\lesssim 10^{-4}$ gauss. Also, the second-order Doppler effect and long wavelength phonons may cause significant line broadening in the laser medium, because of strain-inducing thermal gradients and microscopic scale velocity gradients, respectively, unless the ambient temperature is kept below 10 K.

The line shifts induced by gravitational potential difference across the γ -ray laser medium require that these lasers be operated within an angle $(c^2/gl)(\Delta v/v) \approx 10^{-4}$ rad of the perpendicular to the local gravitational gradient. Likewise,

compared to the stimulated emission cross section. For example, the stimulated emission cross section corresponding to the maximum allowable line width is $\approx 10^2$ barn, whereas the photo-electric cross section may be $> 10^3$ barn. For a laser operating with bandwidth Δv , the cross section for stimulated emission will exceed that for photoelectric absorption only if $Z \lesssim 600k^{1/4} (\Delta v\tau_{\text{spont}})^{-1/4}$. This relation states that, for low photon energies produced by nuclei of high Z , it will be necessary to achieve operating bandwidths approaching the minimum useful widths $\approx \alpha/\tau_{\text{spont}}$. This suggests a figure of merit for nuclear isomeric candidates: $\zeta \equiv \sigma_{\text{max}}/(1 + \alpha)\sigma_{\text{abs}}$ where $\sigma_{\text{max}} = \lambda^2/8\pi$ and σ_{abs} is the sum of the photoelectric and Compton scattering cross sections. This figure of merit is obviously the multiple of the minimum attainable width at which the γ -ray laser will still have a net gain, if the nuclei of the medium are completely population-inverted. Incompletely pure media reduce this figure of merit proportionally to the fractional population inversion. Because ζ is, generally speaking, never very large we are not optimistic about proposals^{7,8} for using isomers with $\tau_{1/2} > 10^6$ s.

Photoelectric absorption and internal conversion lead to heating of the gently pumped γ -ray laser medium that might destroy the recoil-less narrowing of the emission line necessary for laser action. For isomeric states with half lives $> 10^2$ s, however, the heat generated by internal conversion can be easily removed on the time scales over which laser action occurs by thermal conduction cooling, if the radius of the laser medium is ≤ 10 μ m. Heating by photoelectrons in a fibre 1 μ m in diameter resulting from a γ -ray laser pulse of length τ_{pulse} s is of the order of $10^5 k (\sigma_{\text{photo}}/\sigma_{\text{stim}})/\tau_{\text{pulse}}$ eV s⁻¹ per atom, where σ_{stim} is the stimulated emission cross section. A laser medium self-heating rate of 10^2 eV s⁻¹ per atom is acceptable (resulting in operating temperatures of 10K for the medium

and implies that $\sigma_{\text{stim}}/\sigma_{\text{photo}}$ must exceed $10^3 k/\tau_{\text{pulse}}$. Since τ_{pulse} must be shorter than $\tau_{1/2}$, the isomeric state half life, this implies that $\sigma_{\text{stim}}/\sigma_{\text{photo}}$ must be greater than $10^3 k/\tau_{1/2}$. For $\tau_{1/2} > 100$ s, this criterion will be satisfied for $k < 0.1$ if $\sigma_{\text{stim}} > \sigma_{\text{photo}}$, which is the basic net gain condition for γ -ray lasers (as $\alpha \gg 1$). Thus photoelectric self-heating of the medium during laser action is not a serious problem, if $\tau_{1/2} \gtrsim 100$ s.

Because of the difficulties of producing small γ -ray line widths in bulk samples, Goldanskii and his colleagues¹⁷⁻¹⁹ see promise only in pulsed ($\lesssim 10^{-2}$ s) nuclear population inversion, where larger values of Δv would be permitted. While we concur with the Goldanskii-Kagan analysis of the requirements on γ -ray laser action in the half life regime $10^{3.5 \pm 1}$, we see these requirements as attainable through extant laser and semi-conductor technologies, and thus do not share their pessimism in these respects. In contrast, scrutiny of essentially all nuclear data published through 1972 (refs 20, 21 and R. Haight, private communication) does not indicate the existence of even one candidate nuclear species for the nuclear explosion-pumped approach advocated by the Goldanskii-Letokhov school. Such a nucleus must have an excited state half life of $> 10^{-6}$ s (for substantial thermal neutron fluxes cannot be generated through fast flux moderation on shorter time scales) but $\lesssim 10^{-2}$ s (by the Goldanskii assumptions on the required fractional line width), and have a thermal capture cross section of $\gtrsim 10^{-20}$ cm² (to prevent overheating of the laser medium associated with thermal neutron fluences of $\gtrsim 10^{20}$ cm⁻²). Indeed, the ¹⁸¹Ta nucleus nominated for such a role^{17,18} is now known to have several times too small a cross section for neutron capture, though it is the most apt choice we have identified so far. In addition, realistic evaluation of the magnitude of the pulsed time-integrated neutron fluxes to which a medium may be subjected and still show recoil-less narrowing indicates that the estimate of 10^{20} cm⁻² is optimistic by at least a factor of three, when heating by scattering of the incompletely moderated (epithermal) neutron spectrum and other nuclear explosion-associated effects (prompt gamma rays, hydrodynamic and thermal conduction energy transport, for example) are considered. The nuclear explosion-pumped γ -ray laser advocated by Goldanskii and collaborators thus fails of attainability by at least an order of magnitude, in the most favourable case for it.

Realisation of gently pumped lasers

An attempt to operate a γ -ray laser might commence by irradiation of an appropriate target in a high flux nuclear reactor or charged particle accelerator for several nuclear isomeric state lifetimes, so that saturation quantities of isomeric nuclei would be produced. The target might then be rapidly inserted into an atomic beam oven, and the nuclear isomeric state and its daughter ground state nuclei efficiently evaporated into a well collimated atomic beam from which the isomeric state atoms could be routed via any of several photophysical processes (ref. 13, UCRL74904 and ref. 22) into a very small channel (width ~ 1 μ m, length 0.1-1 cm) machined into a heat sink over which the isomeric beam component rather uniformly loaded. The heat sink would then be rapidly levelled, acoustically isolated, magnetically shielded and cooled to liquid helium temperatures, whereupon γ -ray lasing action would commence.

As a particular example, we consider ⁶⁰Co, whose isomer decays in 10.7 min through a 59-keV M3 transition to a long lived (5.2 yr) ground state and which has the notable property of being born substantially population-inverted, because of the relatively large neutron capture cross section into the isomeric state and the comparatively small statistical weight of this state. This isomer can be produced in milligram quantities on minute time scales by (high flux) reactor thermal neutron irradiation of naturally occurring ⁵⁹Co. The ⁶⁰Co and ^{60m}Co can be separated from ⁵⁹Co by γ -cascade-recoil ejection from thin foils at the time of formation. Assembly of the separated isomeric fraction

into a pure, strain-free cylindrical crystal (for example, a metallic cobalt whisker) would be followed by very rapid annealing and purification from impurities generated by β -decay in the panned focal spot of a laser beam, exploiting the very short thermal relaxation times of these whiskers, γ -ray lasing would commence as the required environmental conditions were attained. The relative width that must be achieved to obtain γ -ray laser action for this material is $\sim 3 \times 10^{-20}$.

Some nuclei we consider to be candidates for gently pumped γ -ray laser action are given in Table 2. A more complete list of candidate nuclei has been prepared by L. P. Somerville (UCLL Memo 5574-220). Many variations on this basic theme are obvious, some of which are to be preferred for particular nuclear isomers (for example, those with Z different from that of their parent nuclei, such as may be readily produced with accelerator beams).

Molecular structure

We have briefly reviewed the salient physical conditions limiting γ -ray laser operation, taken note of the two basic types of laser, and described how each type might operate. Such lasers seem to be candidates for producing coherent photon beams with $h\nu \gtrsim 10$ keV, though quite substantial technological challenges must be met in realising them. Operation of γ -ray lasers with $h\nu \gtrsim 100$ keV, however, seems exceedingly difficult. Time-bandwidth limitations force gently pumped lasers to emit their pulses over multi-second time scales; that is, gently pumped γ RLs would be quasi-c.w. sources of extremely coherent and monochromatic radiation with $0.1 \text{ \AA} < \lambda < 1 \text{ \AA}$. It seems clear that such sources will be extremely useful in the basic and applied sciences, particularly in the determination of the structure and composition of large molecules.

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Bacterial mutation affecting T4 phage DNA synthesis and tail production

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A new type of host defective *Escherichia coli* mutant, *hd590*, is described. $T4^+$ infection of this mutant results in (1) an abnormally low rate of T4 DNA synthesis that is constant throughout the latent period; (2) relatively low amounts of specific late T4 proteins; (3) the production of empty T4 heads, but not of tails or tail fibres. Two types of T4 mutants, one in T4 gene 31 and the other near T4 gene 39, overcome the *hd590* block.

INVESTIGATORS examining the role of the host cell in phage production have isolated several host mutants that permit phage adsorption but block virus production. Three principal types of 'host defective', *hd*, cells in which T4 production does not occur have been reported. In the first, T4 DNA synthesis and tail production are normal, but phage capsids do not assemble¹⁻³. T4 heads normally assemble on the host cell inner membrane⁴, and one T4 gene product, P31, is known to affect the association of head proteins with the cell membrane⁵. Some T4 mutants in gene 31 can overcome the block in head assembly in the first type of *hd* bacterium¹⁻³.

The second principal type of *hd* bacterium, a mutant resistant to rifamycin, seems to possess an altered RNA polymerase⁶. T4 infection of this cell results in a slight delay in T4 DNA and late protein synthesis, as well as in a slight delay in the shut-off of host mRNA synthesis. Characteristics of T4 mutants which can overcome this type of *hd* block have not been reported.

The third type of *hd* bacterium blocks the production of functional tail fibres, producing T4 particles which lack the normal complement of long tail fibres⁷. These fibreless phages can be activated by the *in vitro* addition of long tail fibres.

We describe here a new type of *hd* mutant, *E. coli hd590*, which blocks T4 production. After wild-type T4 ($T4^+$) infection of *hd590*, T4 heads are made, but are not filled with DNA. Tails are not made, and the rate of T4 DNA synthesis is abnormally low for the duration of the latent period. Two types of T4 mutants ($T4_{go590}$) able to form plaques with high efficiency on *hd590* have been isolated and characterised. One type of T4 mutant able to overcome the *hd590* block seems to map in gene 31; the other type seems to map in a different region of the T4 genome, near gene 39.

E. coli hd590 and T4 production

Escherichia coli hd590, a nitrosoguanidine-induced mutant of *E. coli* B011', was selected for its inability to function as a normal host for T4 and T6 bacteriophages. Uninfected *hd590* cells grow well in complex media such as tryptone broth. In a minimal medium, however, such as LSTG (described in Fig. 3 caption) or M9 (glucose, salts, phosphate) *hd590* bacteria fail to divide; instead, they form long, fragile filaments. Addition of casamino acids, vitamins, dipeptides, tryptophan or a mixture of deoxynucleosides to the minimal medium did not stimulate

hd590 cell division. Supplementation of minimal medium with both casamino acids ($10 \mu\text{g ml}^{-1}$) and the mixture of deoxynucleosides ($5 \mu\text{g ml}^{-1}$) permitted slow growth and division of *hd590* cells.

More than 90% of the *hd590* bacteria lost their ability to form colonies within 5 min after addition of $T4^+$ particles at an average multiplicity of infection (MOI) of 5 phages per cell. Although $T4^+$ particles attached to and killed *hd590* bacteria with normal efficiency, infection of these bacteria by $T4^+$ particles did not lead to the production of plaque-forming progeny phages (Fig. 1). The block in $T4^+$ production in *hd590* was independent of temperature between 20°C and 43°C . In Fig. 1 and in most subsequent figures, $T4^+$ infections

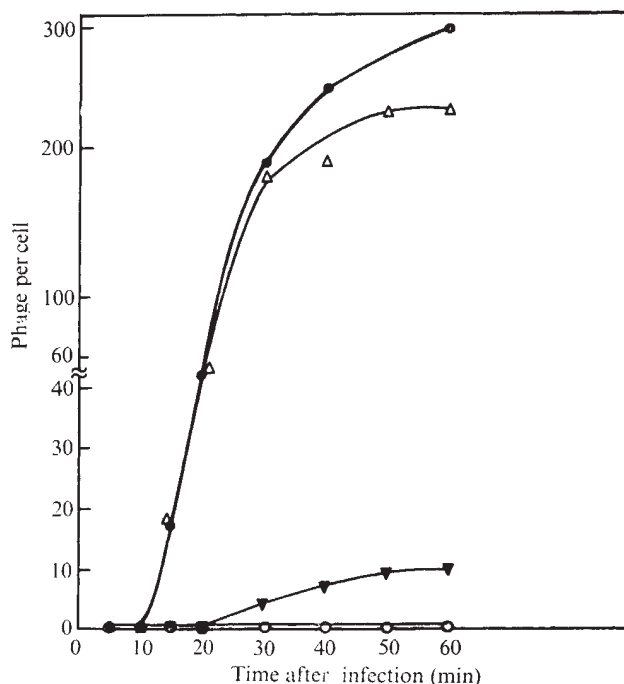


Fig. 1 Phage production and cell killing resulting from T4 infection of *E. coli hd590* and *E. coli* B011'. ●, B011'; $T4^+$; △, B011'; $T4_{go590-1}$; ○, *hd590*; $T4^+$; ▼, *hd590*; $T4_{go590-1}$. Mid-log phase bacteria were used in these and in all other experiments described in this paper. $T4^+$ refers to T4D phages. Bacteria growing in broth (10 g Bacto-tryptone, 5 g NaCl, 1,000 ml H_2O) at 37°C were infected with the appropriate T4 particles (MOI = 5) and 5 min later they were superinfected with the same type of T4 particles (MOI = 5). Viable cell counts were made just before infection and at various times thereafter. Anti T4 serum was added 8 min after infection; 10 min after infection the bacteria were diluted $\times 10^4$ to prevent inactivation of progeny phages by the antiserum. At various times, samples were removed from the infected cultures; they were exposed to chloroform to lyse the bacteria; they were then plated to determine phage production. Both B011' and *hd590* are readily killed by the T4 particles; only B011', however, produces normal yields of T4 particles.

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Fig. 2 Electron micrograph of a negatively stained lysate from $T4^+$ -infected *hd590* bacteria. *E. coli hd590* cells were infected (MOI = 5) and superinfected (MOI = 5) with $T4^+$ particles. One hour after infection chloroform and pancreatic DNase (10 g ml^{-2}) were added to the culture. Samples of the lysate were then negatively stained with 1% phosphotungstate at pH 7.0 and examined in the electron microscope. The lysate contained empty heads (E) polyheads (PS) and polyheads (PH). (Magnification is approximately $\times 55,000$.)

of B011', the parent *E. coli* strain, are shown as control experiments.

Electron microscopic studies of T4 production after $T4^+$ infection of *E. coli hd590* show only empty phage heads, polyheads and polyheads in negatively stained lysates (Fig. 2). Very few tails were observed, and they probably derived from the input phage preparation. The presence of polyheads in the $T4^+$ -infected *E. coli hd590* lysates indicates that at least some tail proteins were made, although the polyheads occurred in a smaller amount than would be expected from a typical T4 mutant blocked in baseplate or tail core assembly.

In agreement with the observations of lysates, electron microscopic examinations of thin sections through $T4^+$ -infected *E. coli hd590* cells showed only empty heads in the central regions of the bacteria. In addition, lumps of head precursor material and τ particles^{4,5} were evident on the bacterial membrane (data not shown). Although empty heads were the predominant head-related structures seen in thin sections, polyheads, resembling tubular τ particles, were also visible. Both polyheads and τ particles, with their long axes extending towards the interior of the cell, appeared to be bound to the cell membrane.

Using sodium dodecyl sulphate (SDS)-acrylamide gel electrophoresis, we analysed the proteins made after $T4^+$ infection of *E. coli hd590*. Normal T4 infection blocks the synthesis of host cell proteins soon after infection; therefore, T4 proteins were specifically labelled with radioactive precursors. Particular gene products were identified by the use of specific T4 amber mutants which block the synthesis of specific proteins. As Fig. 3 shows, bands representing many late T4 proteins

(such as the major head protein, p23 and the tail sheath protein, p18) were present in the $T4^+$ -infected *E. coli hd590* sample; however, bands corresponding to the gene 7 and gene 34 products appeared to be very faint or absent. These gene products are normally parts of the phage baseplate and tail fibre structures, respectively⁶. Several other T4 proteins also seemed to be missing or to be made in relatively small amounts. A few bands not present in the control sample ($T4^+$; *E. coli* B) were seen in the $T4^+$; *E. coli hd590* infection. It is unclear whether these new bands represent fragments of proteins synthesised in normal T4 infections, host proteins or early proteins which have not been turned off.

Since electron microscopy showed that phage heads were assembled but not filled with DNA in $T4^+$ -infected *E. coli hd590* bacteria, we compared the rates of T4 DNA synthesis after phage infection of *E. coli hd590* and *E. coli* B011' at 37° C. The determinations were made by measuring the incorporation of H^3 -thymidine into trichloroacetic acid (TCA)-precipitable material during 2-min pulses given to samples of the infected cultures. As Fig. 4a shows, the rate of DNA synthesis in $T4^+$ infected *E. coli hd590* cells was much lower than the comparable rate in infected *E. coli* B011' cells. The maximal rate of DNA synthesis in $T4^+$ -infected *E. coli hd590* bacteria was approximately 13% of the maximal rate in similarly infected *E. coli* B011'.

Figure 4b shows that the amount of newly synthesised T4 DNA within $T4^+$ -infected *hd590* bacteria was much lower than that in $T4^+$ -infected B011' cells. These data also indicate that DNA synthesis in $T4^+$ -infected *E. coli hd590* began at approximately the normal time, although at a reduced rate which remained constant throughout the latent period (Fig. 4a and b).

In other experiments (data not shown) we found that the DNA which accumulates in $T4^+$ -infected *hd590* cells sediments more rapidly than the 63S DNA from mature T4 particles. This rapidly sedimenting DNA is characteristic of certain T4 infections in which head filling is blocked¹⁰.

In an effort to find reagents which could promote T4 production in *hd590* cells, we observed that the addition of low concentrations of EDTA to $T4^+$ infected *hd590* samples significantly increased the yield of T4 particles (Fig. 5). We added 0.0002 M EDTA to mid-log phase *E. coli hd590* cells in tryptone broth at the time of infection with $T4^+$ particles and found a marked increase in the production of progeny phages. This result was essentially the same whether the EDTA was added at the time of infection, 2 min before infection or 8 min after infection. When we increased the EDTA concentration to 0.1 M or higher, we observed no increase in phage production, presumably because the infected cells were damaged by the EDTA.

We also observed that addition of EDTA could alter the level of DNA synthesis in $T4^+$ -infected *hd590* and B011' cultures (Fig. 5). Although 0.0002 M EDTA had little, if any, detectable effect on DNA synthesis in either sample, higher concentrations of EDTA markedly reduced T4 synthesis in the B011' controls and markedly increased T4 DNA synthesis in the *hd590* samples. Thus, the $T4^+$ -infected *hd590* and B011' samples showed nearly the same levels of DNA synthesis when exposed to 0.0001–0.005 M EDTA.

T4 mutants

T4 mutants (*go590*) able to plate on *E. coli hd590* occur spontaneously at a frequency of about 10^{-8} . One such phage mutant, designated T4go590-1, was studied in detail. We investigated the physiology of a T4go590-1 infection of *E. coli hd590*. Although $T4^+$ -infected *E. coli hd590* cells yielded an average of less than 0.01 progeny phage per cell, T4go590-1-infected *E. coli hd590* cells yielded an average of 10 phage particles per bacterium (Fig. 1). This burst size, although sufficient to cause formation of plaques, is lower than would be expected of a completely normal infection. Negative staining of *hd590* cells lysed by chloroform 20 min after T4go590-1 infection showed a pattern similar to that seen 20 min after $T4^+$ -infection of the cells, with empty heads, polyheads and polysheath. At

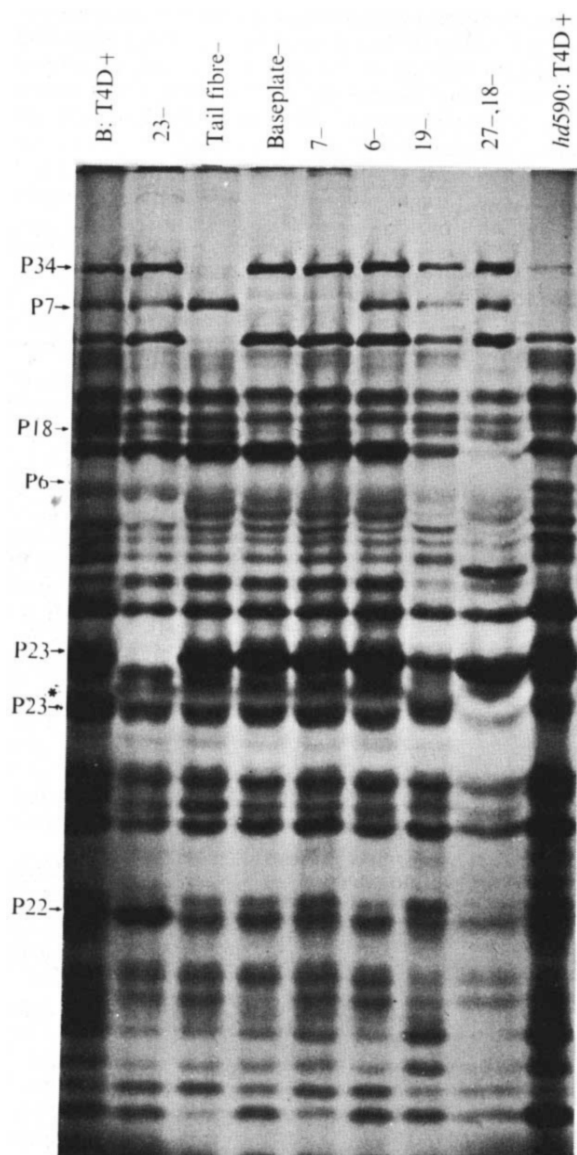


Fig. 3 Autoradiograph of an SDS-acrylamide gel showing the phage proteins in $T4^+$ -infected *hd590* cells. Since *E. coli hd590* (su^+) do not grow well in minimal medium, *hd590* and *E. coli B* (su^+) were grown in tryptone broth to 4×10^8 cells ml^{-1} ; the bacteria were pelleted and resuspended in LSTG medium (6.4 ml 0.1 M KH_2PO_4 , 1.6 ml 0.1 M Na_2SO_4 , 2.0 ml 0.5 M $MgCl_2$, 100 ml $10 \times$ low salts, 100 ml 1.0 M Tris, pH 7.4, 100 ml 20% glucose, H_2O to 1,000 ml; $10 \times$ low salts contains 5.4 g NaCl, 3.0 g KCl, 11.0 g NH_4Cl , 10 ml 0.1 M $CaCl_2$, 10 ml 0.1 M $FeCl_3$, H_2O to 1,000 ml) containing 1% (v/v) tryptone broth. The two cultures were then infected (MOI = 3) and superinfected (MOI = 3) 7 min later with $T4^+$ particles. A mixture of amino acids— ^{14}C (100 μCi ml^{-1} for *hd590* and 20 μCi ml^{-1} for *B*) and methionine— ^{35}S (100 μCi ml^{-1} for *hd590* and 20 μCi ml^{-1} for *B*) was added to the cultures 12 min after infection. The bacteria were pelleted 35 min after infection; the pellets were heated to $100^\circ C$ for 2 min in 1% SDS and 1% β -mercaptoethanol. Other *E. coli B* cultures, growing in LSTG medium, were infected with various *T4* amber mutants and were then treated in the same way as the previously described *E. coli B* sample. The amber mutants used for this gel were *T4amH11* (23 $^-$), *T4amXF18* (baseplate $^-$), *T4amX4E* (tail fibre $^-$), *T4amN102* (6 $^-$), *T4amB16* (7 $^-$), *T4amN120amE18* (27 $^-$, 18 $^-$) and *T4amE1137* (19 $^-$). The denatured proteins were run on SDS-acrylamide (10%) slab gels⁸ for 5.5 h at 80 V, after which the gels were dried. Autoradiographs were then made to allow visualisation of the protein bands. Columns labelled *BT* 4^+ and *hd590:T4* 4^+ indicate *E. coli B* and *E. coli hd590* respectively infected with $T4^+$ phages. All other columns are infections of *E. coli B* by the appropriate *T4* amber mutant.

later times, however, intact phage particles appeared in the *T4go590-1*-infected samples, but not in the $T4^+$ -infected *E. coli hd590* samples.

To determine whether the pattern of phage DNA synthesis in *T4go590-1*-infected *E. coli hd590* differs from that observed after $T4^+$ infection, we measured thymidine incorporation into TCA-precipitable material at various times after the infection of cultures of *E. coli hd590* bacteria with either *T4go590-1* or $T4^+$ phages. The data from this experiment (Fig. 4a) suggest that until 26 min after infection, the rates of DNA synthesis in *T4go590-1* and in $T4^+$ -infected *E. coli hd590* bacteria are similar. After 26 min, however, the rate of phage DNA synthesis in the *T4go590-1* infected culture started to increase whereas there was no comparable increase in the $T4^+$ -infected *E. coli hd590* culture. Sixty minutes after infection, the rate of DNA synthesis in the *T4go590-1*-infected *hd590* culture is about double the rate in the $T4^+$ -infected *hd590* culture.

The map position of *T4go590-1* was determined from analysis of a series of crosses performed as described before¹¹. Genotypes of the progeny phages from such crosses were identified using method II of Doermann and Boehner¹², but adopting modifications appropriate to the particular mutants involved (details in legend for Table 1). The first cross used *T4go590-1* as one parent and, as the second, a strain carrying mutations in the following 12 genes: *e*, 5, 11, 24, 48, 34, 37, *ac*, *rIIB*, 39, 43, and 46. Data from more than 1,000 progeny phages showed that the recombination frequency of *go590-1* with other markers was lowest with *amN85* (gene 48) and increased progressively for markers in both the clockwise and the counterclockwise directions from gene 48. The maximum value was attained with *amN130* (gene 46). In cross 2 therefore, the second parent was replaced by a phage with markers in genes 25, 26, 51, 27, 29, 48, 31 and 34. Although 1,050 progeny were scored and both complementary recombinants found with all other markers, not a single recombinant was found for *go590-1* and *amN54N* (gene 31). Cross 3 involved parents with markers in 7 genes (29, 48, 30, 31, 32 and 34), and analysis of 1,118 progeny phages again failed to uncover a single recombinant between *go590-1* and *amN54* although all other recombinant classes were recovered with reasonable frequencies. Thus, among more than 2,000 cross-progeny phages neither the doubly mutant nor the wild-type recombinant for these two markers was found. It must be concluded that *go590-1* is very close to *amN54* in gene 31 or perhaps represents a multisite mutation whose ends overlap the site of the *amN54* mutation.

With the possibility of a multisite mutation in mind, recombination between *go590-1* and a second amber mutation in gene 31 (*amNG71*, supplied by W. B. Wood) was examined. Both wild-type and doubly mutant recombinants were found. The cross was repeated with the mutant markers in coupling. The marker *amH39* (gene 30) was also included in both crosses in the hope of ordering the mutations by three-point tests. Parallel crosses were made with *go590-1* replaced by *amN54*. The results of the four crosses are summarised in Table 1. If *go590-1* is due to a multisite mutation it might be anticipated that recombination between it and *amNG71* is less than between *amN54* and *amNG71* because the effect of a multisite mutation might reduce recombination beyond *amN54* in either direction. The results show no evidence for that hypothesis. We also note that the data suggest that the order of markers is that used in Table 1, namely *amH39-amN54* (or *go590-1*)-*amNG71*. High negative interference reduced the effectiveness of the three-point test here as in other cases of close linkage¹¹ so that the ordering of the markers in these crosses is less than completely certain.

Four *T4* mutants of independent origin able to grow on *hd590* were isolated by Barbara North and Gary Thorgaard, who permitted us to comment here on their results. Each of these strains simultaneously acquired the ability to multiply also on the bacterial mutant *hdF* isolated by Revel¹³. All of these strains differ from *go590-1* which is unable to make plaques on *hdF*. H. R. Revel (personal communication) has shown that her *T4goF*

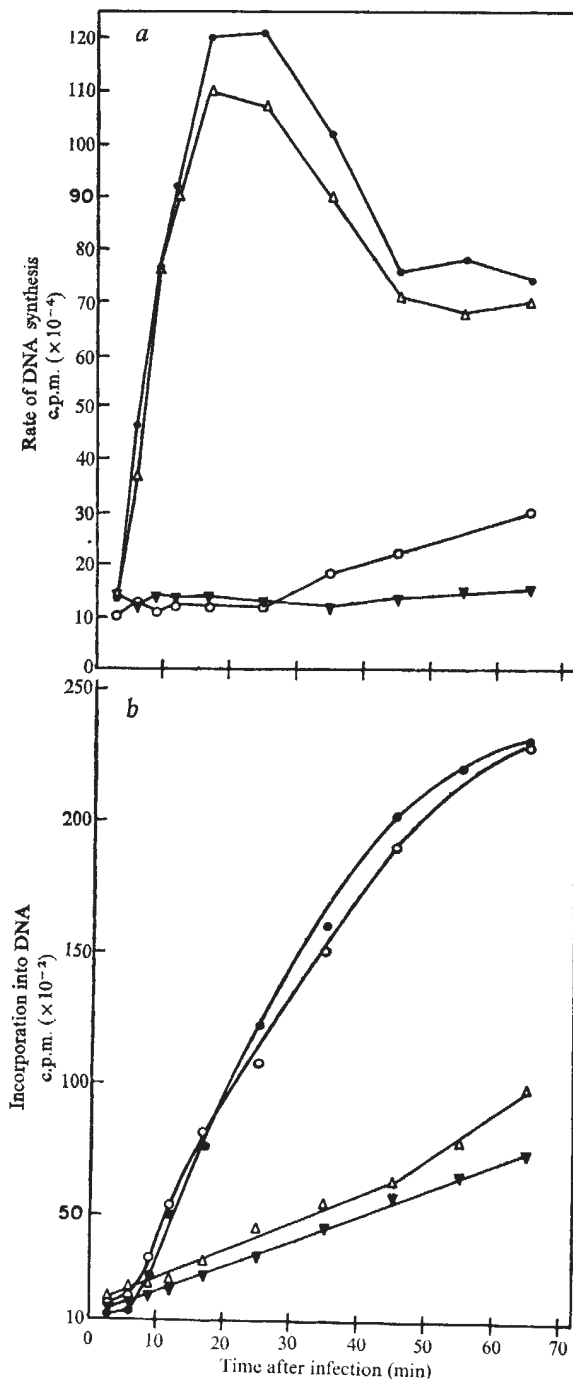


Fig. 4 Synthesis of DNA after T4 infection of *hd590* and B011'. Bacteria were grown to 5×10^8 ml⁻¹ at 37° C in tryptone broth which contained 100 μ g ml⁻¹ deoxyadenosine and 0.5 μ g ml⁻¹ thymidine. The cells were then infected with the appropriate T4 particles (MOI = 5) and 7 min later they were superinfected (MOI = 5) with the same type of phage. *a*, To measure rates of DNA synthesis, at various times after infection, 1 ml samples were removed from the culture; they were added to 10 μ Ci ³H-thymidine. Two minutes later ice-cold TCA plus thymidine was added to a concentration of 10% TCA, 50 μ g ml⁻¹ thymidine. After 10 min the material insoluble in the cold 10% TCA was collected on glass fibre filters which were washed twice with 5% TCA containing 25 μ g ml⁻¹ thymidine and then twice with ethanol. The filters were dried and counted in a liquid scintillation counter. Δ , B011': T4⁺; $\bullet$, B011': T4^{go590-1}; ∇ , *hd590*: T4⁺; $\circ$, *hd590*: T4^{go590-1}. *b*, To measure total TCA-precipitable DNA ³H-thymidine (10 μ Ci ml⁻¹) was added to the bacterial cultures at the time of infection. At various times thereafter 10 μ l samples were placed on filters which were then immersed in ice-cold 10% TCA. The filters were then treated as described in (*a*). $\bullet$, B011': T4⁺; $\circ$, B011': T4^{go590-1}; ∇ , *hd590*: T4⁺; Δ , *hd590*: T4^{go590-1}.

mutants map in the vicinity of gene 39. One of the new *go590* mutants has been shown to map far from gene 31 and near gene 39, having yielded 4.4% recombinants with *amS2* (gene 39). It seems clear that either a mutation in gene 31, a gene essential for capsid development, or a mutation in another region separated from gene 31 by one-fourth of the T4 chromosome, can compensate for the alteration of *hd590* which prevents wildtype T4 from multiplying in it.

Implications

The data presented here describe a host defective bacterium, *E. coli* *hd590*, in which the production of T4⁺ phages is blocked. Unlike other T4 *hd* bacteria that have been reported^{1-3,6,7}, T4⁺-infected *hd590* cells show: (1) an abnormally low rate of phage DNA synthesis, a rate which is constant throughout the latent

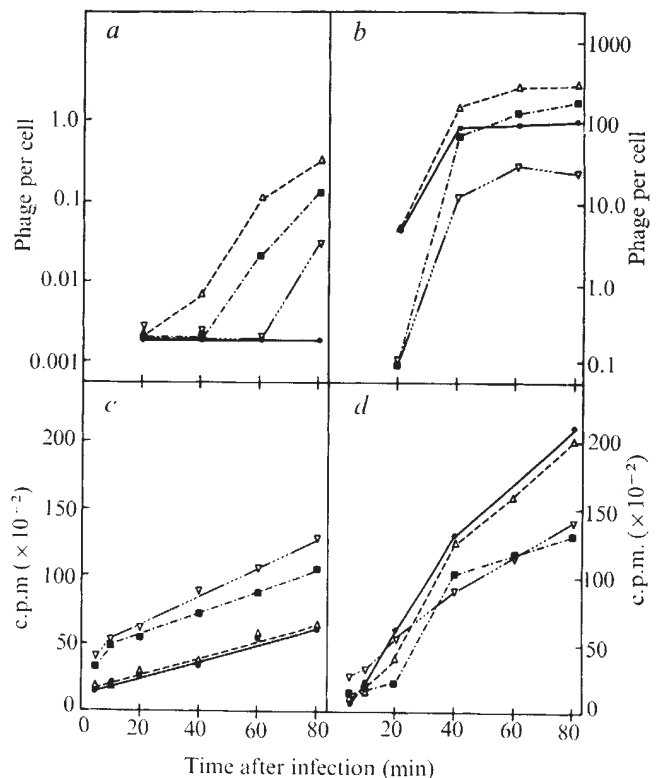


Fig. 5 Effect of EDTA on T4⁺ production in *hd590* and in B011'. Bacteria were grown to 5×10^8 cells ml⁻¹ at 37° C in tryptone broth which contained 100 μ g ml⁻¹ deoxyadenosine and 0.5 μ g ml⁻¹ thymidine. EDTA was then added to the appropriate final concentration and the cells were infected with T4⁺ particles (MOI = 3); 5 min later the cells were superinfected to inhibit lysis with additional phages (MOI = 3). In experiments to measure phage production, anti T4 serum was added to the cultures 10 min after infection to inactivate unadsorbed T4 particles, and 5 min later the cultures were diluted 10³-fold to prevent inactivation of progeny phages. At various times thereafter, samples were withdrawn from the cultures, lysed with chloroform, and assayed for plaque-forming T4 particles. Experiments to measure DNA synthesis were performed as described in Fig. 4b. *a*, Phage production in *hd590*; *b*, phage production in B011'; *c*, DNA synthesis in *hd590*; *d*, DNA synthesis in B011'. $\bullet$, No EDTA; Δ , 0.2 mM EDTA; $\blacksquare$, 1.0 mM EDTA; ∇ , 5.0 mM EDTA.

period; (2) relatively small amounts or the absence of specific late T4 proteins; (3) empty heads, but no tails or tail fibres, in lysates. The absence of tails and tail fibres is probably due to the absence of the products of genes 7 and 34 in T4⁺-infected *hd590* samples, gene products that are parts of the T4 baseplate and tail fibre, respectively⁹. Two types of T4 mutants, one which appears to map near T4 gene 39 and one which appears to map

Table 1 Location of *go590-1* in the T4 genetic map

Progeny genotypes	Cross 1	Cross 2	Progeny genotypes	Cross 3	Cross 4
<i>am₁am₂am₃</i> + + +	5 12	58 95	<i>am₁ + am₃</i> + go +	7 20	51 79
<i>am₁am₂ +</i> + + <i>am₃</i>	508 567	1* 5*	<i>am₁ + +</i> + go <i>am₃</i>	438 595	3* 4*
<i>am₁ + +</i> + <i>am₂am₃</i>	5* 1*	446 577	<i>am₁ go +</i> + + <i>am₃</i>	9* 2*	551 487
<i>am₁ + am₃</i> + <i>am₂ +</i>	52 39	4 4	<i>am₁ go am₃</i> + + +	57 61	6 6
Total phages identified	1,189	1,190		1,189	1,187

Crosses were made in *E. coli* CR63 following the methods outlined by Chase and Doermann¹¹. The genotypes of individual progeny phages were identified as described by Doermann and Boehner¹², but with the following technical modifications to accommodate peculiarities of specific mutants. The wild-type and mutant alleles of *go590-1* were distinguished by replicating on to a test plate in which the 5ml soft-agar overlay contained 2.5×10^7 wild-type T4 per ml, 0.4 ml of a 20:1 mixture (v/v) of aerated overnight broth cultures of *hd590* and CR63/s, and streptomycin (*hd590* is resistant to streptomycin, having been derived from the streptomycin-resistant strain 011'). To differentiate *amH39* from its wild-type allele a streptomycin-resistant mutant was isolated from the *E. coli* strain *lop8lig2* isolated by Gellert and Bullock¹⁴ and supplied by Hillard Berger. The soft agar overlay of the replica plates contained, in addition to streptomycin, 10^7 *amH39* phage per ml as well as 0.4 ml of a 59:1 mixture (v/v) of overnight cultures of *lop8lig2*/s and CR63/s. The *amNG71* tests were made using streptomycin, a 20:1 mixture of B/s and CR63/s, and 2.5×10^7 *amNG71* per ml soft agar. In the Table, *am₁*, *am₂*, and *am₃* represent *amH39*, *amN54*, and *amNG71* respectively. Asterisks mark the classes in each cross presumed to be double crossovers on which the order of the markers has been based. The parental classes are, of course, the most frequent pair.

in T4 gene 31, can overcome the *hd590* block. T4 gene 39 seems to function to promote normal T4 DNA synthesis¹⁵; T4 gene 31 has been thought to function specifically in T4 head assembly⁸. The 31 mutant, T4*go590-1*, is different from those other mutants in gene 31 which are able to produce phage in the *hd* cells which block T4 head morphogenesis¹⁻³. This is the first report, to our knowledge, of a T4 gene 31 mutant which affects the synthesis of T4 DNA and late proteins.

Although we could not transfer the *hd590* character into another cell, two lines of evidence suggest that the block in T4 production results from a single host cell mutation. First, mutant T4 phages, such as T4*go590-1*, which can overcome the *hd590* block appear to be mutated in a single gene. Therefore, one specific phage alteration can affect T4 DNA synthesis, protein synthesis and head filling in *hd590*. Second, preliminary studies suggest that among bacterial revertants the various aspects of the *hd590* character are lost jointly, as would be expected if the *hd590* phenotype were due to a single mutation.

If the *hd590* character results from a single alteration of the host cell, what is its molecular basis? The low rate of T4 DNA synthesis in *hd590* cells does not seem to result from a failure to synthesise T4 DNA polymerase (gene 43 product¹⁶). In extracts of T4⁺-infected *hd590* cells, we find normal levels of T4 polymerase activity *in vitro* (unpublished data of D. S., B. Fansler and L. D. S.). Furthermore, sedimentation profile analysis (unpublished data of McLaughlin and L. D. S.) indicates that the apparent low rate of DNA synthesis in T4⁺-infected *hd590* cultures is not due to degradation of the T4 DNA.

In normal T4⁺ infection, late mRNA production is coupled with DNA synthesis¹⁷. Since T4 DNA synthesis is abnormal in *hd590*, the synthesis of late mRNA may also be abnormal, and, if so, may be responsible for the reduced synthesis of specific late T4 proteins in T4⁺-infected *hd590* cells. Alternatively, the *hd590* defect might specifically prevent assembly of tail fibres and baseplates, thereby leaving the products of genes 7 and 34

unassembled, and sensitive to proteolytic activities within the infected cell. It seems unlikely that the absence of various proteins from T4⁺-infected *hd590* cells is simply due to an increased host cell protease activity, since T5 and T7 phages plate with normal efficiency on *hd590*.

Available evidence suggests that the altered host cell component in *hd590* may be associated with its inner membrane. This suggestion is based on two observations. (1) Low concentrations of EDTA, which primarily seem to affect the bacterial membrane¹⁸, stimulate T4⁺ DNA synthesis and phage production in *hd590*. (2) A specific T4 mutant in gene 31, T4*go590-1*, can overcome the *hd590* block in T4 production. T4 gene 31 product normally functions in the assembly of the T4 capsid, at the level of cell membrane interaction with T4 head proteins^{4,5}. Thus, either EDTA or a specific mutant T4 gene 31 product, both of which may interact with the host cell inner membrane, can stimulate T4 DNA synthesis and phage production in *E. coli* *hd590*.

Georgopolous and Herskowitz¹⁹ have described *hd* mutants called GroP⁻ which affect λ DNA synthesis. Specific λ mutants in gene P can overcome the GroP⁻ block, presumably by making an altered P product that can interact with the altered host cell component. The GroP⁻ defect has been traced to an alteration of the DNA 'B' gene of *E. coli*¹⁹. The low level of T4 DNA synthesis in *hd590* cells might also be due to an alteration of the bacterial DNA synthesis machinery. This suggestion would imply a greater dependence of T4 on the *E. coli* DNA replication system than has heretofore been assumed, since T4, in contrast to λ , codes for more than 20 phage-induced enzymes of DNA metabolism²⁰—including DNA polymerase¹⁶, polynucleotide ligase²¹, polynucleotide kinase²² and various deoxyribonucleases²³.

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Evolution of C-type viral genes: inheritance of exogenously acquired viral genes

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Genes related to the nucleic acid of an endogenous domestic cat C-type virus (RD114) are found in the cellular DNA of anthropoid primates while many members of the cat family Felidae lack these sequences. Endogenous viruses from primates are thus concluded to have infected and become part of the germ line of an evolutionarily distant group, the ancestors of the domestic cat.

We have evidence that C-type viruses have in the past been transferred between vertebrate species that are only remotely related phylogenetically. Our data suggest that viral genes from one group of animals can give rise to infectious particles that not only can integrate into the DNA of another species, but can also be incorporated into the germ line and be transmitted as cellular genes. We have found that the RD114/CCC group¹⁻³ of endogenous feline viruses originated from a primate C-type virus horizontally transmitted to some species of *Felis* relatively recently in feline evolution.

The chromosomal DNA of various mammalian species contains multiple copies of nucleic acid sequences that can code for the production of endogenous C-type viral particles⁴. With ³H-DNA transcripts prepared from the endogenous baboon C-type viruses^{5,6}, partially homologous sequences have been detected in the cellular DNA of Old World monkeys, apes and man^{7,8}. These virogenes have evolved for at least 30 Myr among the anthropoid primates in a fashion that correlates closely with the taxonomic relatedness of the species⁸. Domestic cat DNA contains C-type virogenes⁹⁻¹² which can give rise to RD114/CCC viruses^{2,3}. By several criteria, including nucleic acid sequence homology^{5,7} and antigenicity of the polymerase and the p30 protein^{6,13,14}, these viruses are related to, but distinct from, the baboon viruses. The unexpected relatedness of the endogenous cat and the endogenous baboon viruses suggested that they had a common origin.

RD114-related sequences in cats and primates

The origin of the RD114/CCC cat viruses can be clarified by examining the cellular DNAs from other members of the Felidae, which consist of 36 species belonging to six genera. The use of labelled unique sequence (non-repetitive) cellular DNA to compare the extent of nucleotide sequence divergence among species has shown that the cellular DNAs of the species examined have a nucleic acid sequence homology consistent with classical phylogeny¹⁵. Hybridisation of labelled unique-sequence domestic cat DNA to the DNA of other Felidae (Fig. 1b) showed that they are all closely related. The DNA of another carnivore (dog) contains much less sequence homology to domestic cat cellular DNA. All modern carnivores are believed to have had a common ancestor approximately 35 Myr ago; the close relationship observed between the DNA of all members of the Felidae indicates that they represent a more recent radiation (<15 Myr ago). The cellular DNA of the cats examined thus contains nucleic acid sequences closely related to each other, as are those of the Old World monkey subfamily Cercopithecinae⁸. The Felidae and the Cercopithecinae, however, lack detectable reciprocal DNA sequence homology.

Hybridisation of cat cellular DNAs to a ³H-DNA transcript prepared from RD114 virus (Fig. 1a) showed that homology was greatest with the domestic cat (*Felis catus*) DNA; at the maximal extent of the reaction, approximately 80% of ³H-DNA was resistant to digestion with the single-strand specific nuclease, S₁. The cell DNA from three other species of cats, European wildcat (*F. sylvestris*), sand cat (*F. margarita*) and jungle cat (*F. chaus*), also contained nucleic acid sequences homologous to the RD114 ³H-DNA probe. Under our hybridisation conditions, however, no other species of *Felis* or any other feline genus had base sequences related to RD114. In contrast, the DNA of all primate species whose unique sequence cellular

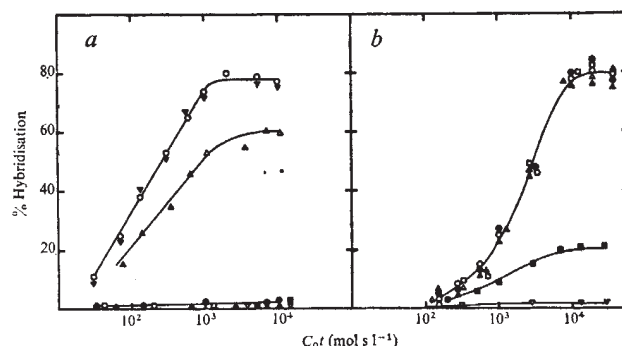


Fig. 1 Hybridisation of RD114 C-type viral ³H-DNA probe and domestic cat unique sequence cellular ³H-DNA to feline cellular DNAs. The ³H-thymidine-labelled DNA probes were synthesised from detergent-disrupted C-type virus in the presence of actinomycin D as described¹⁹. The specific activity of the ³H-DNA was 1.8×10^7 c.p.m. μg^{-1} . The ³H-DNA probes contained 63–72% of their respective 70S viral RNA sequences at a ³H-DNA: ³²P-viral RNA molar ratio of 1.5 (ref. 7), indicating that they contain most of the sequences present in viral RNA and that these sequences are in proportions similar to their content in 70S RNA. Cellular DNA was extracted from tissues and cell lines as described⁷. All DNAs were sonically treated so as to yield a mean size of 6–8S (the size of the ³H-DNA probes) as determined by centrifugation on alkaline sucrose gradients⁷. DNA:DNA hybridisations were incubated at 65°C in reaction mixtures containing 0.01 M Tris, pH 7.4, 0.75 M NaCl, 2×10^{-3} M EDTA, 0.05% SDS, 10,000 to 20,000 c.p.m. of ³H-DNA and 1–3 mg of cellular DNA per ml. Hybridisations were started by heating the mixtures to 98°C for 10 min, cooling on ice to 4°C and incubating at 65°C. At various times (from 15 min to 96 h), 0.05 ml portions were removed and frozen at –80°C until digested with the single-strand-specific nuclease, S₁, as described¹⁹. C₀t values (C₀ is the concentration of cellular DNA in mol l⁻¹, and t is the time in seconds) were calculated as suggested by Britten and Kohne²⁰ as $(A_{260} \text{ ml}^{-1}) / 2 \times h$, and corrected to a monovalent cation concentration of 0.18 M²¹. a, Annealing of ³H-DNA probe prepared from RD114 grown in RD cells¹ to DNA extracted from: (○) domestic cat kidney; (▼) European wildcat liver; (△) jungle cat liver; (●) leopard cat spleen; (□) tiger liver; (▲) lion spleen; (■) dog liver; (▽) human spleen. b, Annealing of unique sequence domestic cat cellular ³H-DNA to the cellular DNA of various cats. The DNA of a domestic cat cell line was labelled with ³H-thymidine and extracted⁷. Unique sequence cellular DNA was isolated by removing the highly reiterated sequences (approximately 35% of the total DNA) that anneal by a C₀t of 100 by fractionation on hydroxylapatite⁷. This ³H-thymidine-labelled unique sequence DNA was then hybridised to the DNAs listed above. Symbols are the same as in (a).

DNA is homologous (or nearly so) to baboon cellular DNA hybridised extensively to the baboon C-type virus probe^{7,8}.

The thermal stability of nucleic acid hybrids is an index of base-pair mismatching between the strands of a double-strand DNA molecule. Mismatching yields hybrids that melt at a lower temperature; the overall effect of mismatched base-pairs on the thermal stability is approximately 1° C for 1% altered base-pairs¹⁶. Thermal stability data obtained with 13 species representing six feline genera by using either labelled non-repeated domestic cat cellular DNA or ³H-RD114 viral DNA as a probe are shown in Table 1. Although the overall unique-sequence cellular DNAs of all the cat species were highly homologous (the largest difference in thermal stability is less than 2° C), only four species of *Felis* contained RD114-related sequences. There were no sequences related to RD114 ³H-DNA in two carnivore species (mink and dog) or in mouse or cow cell DNA. Nucleic acid sequences related to RD114 were found in six species of Old World monkeys. Among the apes,

sequences related to RD114 or CCC ³H-DNA probes can be detected readily in chimpanzee and gorilla cellular DNA. For reasons not yet clear, greater homology has been observed consistently with these two apes as compared with gibbon and human DNA. Sequences related to the baboon C-type virus, while found in all apes, are also detected more readily in chimpanzee and gorilla DNA⁸. Human DNA has been examined for RD114-related nucleic acid sequences with negative results⁹⁻¹². The lack of detectable sequence homology of human DNA with RD114 is not due to an absence of RD114-related information. In support of this conclusion, p30 protein partially related to RD114 p30 can be found in tissues of humans¹⁷, as well as baboons¹³ and other Old World monkeys¹⁸.

Melting curves obtained with a single-stranded ³H-DNA transcript of RD114 RNA hybridised to the DNA of domestic and jungle cat and to the DNA of Old World monkeys are shown in Fig. 2a. The RD114-related sequences in these

Table 1 Nucleic acid homology and thermal stability between domestic cat unique sequence cellular DNA and RD114 viral DNA and the DNA from various species

Species*	Geographical range	Unique sequence domestic cat Cellular ³ H-DNA†		RD114/CCC viral ³ H-DNA§	
		% hybrid	ΔT _m	% hybrid	ΔT _m
Carnivores					
Felidae					
Felis					
domestic cat (<i>F. catus</i>)	North Africa†	100	<0.5	100	<0.5
European wildcat (<i>F. sylvestris</i>)	Europe, Asia Minor	>95	<0.5	95	<0.5
sand cat (<i>F. margarita</i>)	North Africa, Arabia	>95	<0.5	81	<1.0
jungle cat (<i>F. chaus</i>)	Nile Valley, Asia Minor	>90	<1	70	1.5
leopard cat (<i>F. bengalensis</i>)	Southeast Asia	>90	<1	<2	—
golden cat (<i>F. temmincki</i>)	Southeast Asia	>90	<1	<2	—
geoffroy's cat (<i>F. geoffroyi</i>)	South America			<2	—
Lynx					
caracal cat (<i>L. caracal</i>)	Africa, Asia Minor	>90	<1	<2	—
bobcat (<i>L. rufus</i>)	North America	>90	<1	<2	—
Panthera					
lion (<i>P. leo</i>)	Africa, Asia	>90	<1	<2	—
tiger (<i>P. tigris</i>)	Asia			<2	—
Leo					
leopard (<i>L. pardus</i>)	Africa, Asia	>90	<1	<2	—
Uncia					
snow leopard (<i>U. uncia</i>)	Central Asia	>90	<2	<2	—
Acinonyx					
cheetah (<i>A. jubatus</i>)	Africa, Asia Minor			<2	—
Other carnivores					
mink		23	10	<2	—
dog		19	11	<2	—
Primates					
New World monkeys (<i>Cebidae</i>)					
capuchin		2	—	<2	—
Old World monkeys (<i>Cercopithecoidea</i>)					
baboon		2	—	20	10.5
mangabey		3	—	15	11.0
patas		2	—	11	11.0
African green		3	—	11	11.0
macaque: rhesus		1	—	18	11.0
stump-tail				17	10.5
Apes and man (<i>Hominoidea</i>)					
chimpanzee		2	—	7.5	NT
gorilla		2	—	8.5	NT
gibbon		1	—	<2	—
human		1	—	<2	—
Other mammals					
mouse		2	—	<2	—
cow		2	—	<2	—

* Cellular DNA was extracted from various tissues of the species listed.

† Since its domestication this cat has travelled with man all over the world. All breeds of the domestic cat (for example, Burmese, Siamese and Abyssinian) belong to the same species.

‡ ³H-thymidine-labelled unique sequence domestic cat cellular DNA was hybridised to the cellular DNA of the various species as described in Fig. 1. The % hybrid is the saturating normalised value obtained after digestion of the hybrids with S₁ nuclease. The actual final extent of domestic cat cell DNA hybridisation to the cellular ³H-DNA probe was 73%. The temperature at which 50% of the hybrids are dissociated (T_m) was 85° C for the homologous domestic cat: domestic cat hybrid; the ΔT_m is the difference in T_m between the other DNA:DNA hybrids and the T_m of the homologous hybrid. The T_m of hybrids that hybridise less than 3% cannot be determined because of insufficient homology.

§ A ³H-DNA probe prepared from RD114 virus grown in a human cell line was used for the hybridisations to feline DNA, and a ³H-DNA probe prepared from CCC virus grown in a canine thymus cell line (FCf2Th) for the hybridisations to primate DNA. The T_m of the homologous RD114: domestic cat DNA hybrid was 88.5° C.

NT, not tested.

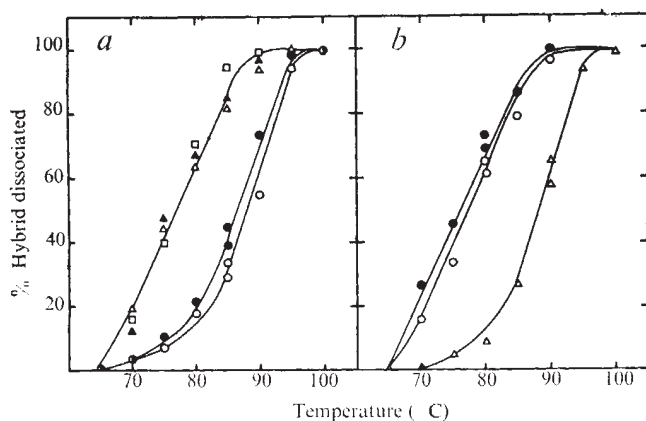


Fig. 2 Thermal stability of hybrids formed between baboon viral ^3H -DNA and RD114 ^3H -DNA and various feline and primate cellular DNAs. *a*, An RD114 ^3H -DNA probe was hybridised to DNA extracted from: (○) domestic cat liver; (●) jungle cat spleen; (△) baboon lung; (▲) patas liver; (□) rhesus liver. *b*, A baboon C-type ^3H -DNA probe was hybridised to DNA extracted from: (△) baboon lung; (○) domestic cat liver; (●) jungle cat spleen. After hybridising to a C_0t of 10^4 , aliquots (containing 400 to 700 c.p.m.) were heated for 5 min in 0.75 M NaCl at the indicated temperatures. The amount of hybrid remaining was determined with the single-strand-specific nuclease, S_1 .

primates melt at approximately 11°C lower than the homologous hybrid. The lower thermal melting points obtained with the Old World monkey DNAs indicate that the partial homology detected is not the result of a strict preservation of discrete continuous segments of the viral genomes in these species, but rather of a more general base-pair substitution throughout the genome. Figure 2*b* shows the reciprocal experiment: the melting curves obtained when a single-stranded ^3H -DNA transcript of baboon C-type viral RNA was hybridised to the DNA of baboon and domestic and jungle cat. Again, baboon type C related sequences in the cellular DNA of the two cats melt at about 11°C lower than the homologous hybrid.

Models for viral transmission among species

When the virogenes of two species are more closely related to each other than are the cellular genes, one must suspect horizontal transmission and subsequent perpetuation of the viral genes through the germ line. Figure 3 shows models which could account for the data. A virus of unknown origin infects the ancestors of certain cats and primates in model *a*. In model *b*, a virus ancestral to the four species of cats described above infects an ancestor of the Old World monkeys and apes. In model *c*, the converse occurs: an endogenous primate virus infects a recent ancestor of the domestic cat. If RD114 were an endogenous Felidae virus, there should be sequences related to it in the cellular DNA of all Felidae. Since RD114-related nucleic acid sequences can be found in all Old World monkey and ape tissues but in just four closely-related species of Felidae, we conclude that RD114 is a primate C-type virus, as shown in model *c*. The greater homology to RD114 nucleic acid sequences in Old World monkey cell DNA than in ape DNAs (Table 1) shows that the transmission occurred after the Old World monkeys and the apes diverged (arrow II or III). Since all the Old World monkey DNAs show comparable levels of homology to RD114, model cII is most likely.

The cats of the genus *Felis* that contain DNA sequences related to RD114 are from the Mediterranean basin (Table 1) while those from south-east Asia and the New World lack these sequences. The larger African cats (leopard and lion) also do not contain RD114 sequences in their cellular DNA. Therefore, the successful transmission of gene sequences was subsequent

to the major radiation of Felidae and limited to one geographic region.

C-type viruses have thus under natural conditions transferred genetic information between species that are only remotely related phylogenetically. We have shown here that viral genes from one group of animals can give rise to infectious particles that not only can integrate into the DNA of animals of another species, but can also be incorporated into the germ line. There may well be other examples among C-type viruses of germ line inheritance of exogenously acquired viral genes.

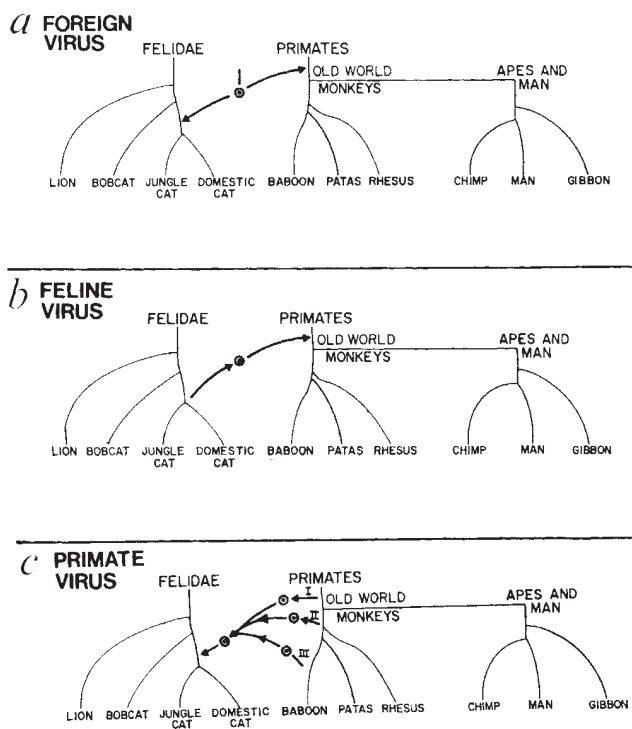


Fig. 3 Schematic representation of models which could account for the presence of related virogenic information in primates and domestic cats. *a*, Common foreign virus infection of ancestors of both Old World monkeys and domestic cats. *b*, Infection of the ancestors of Old World monkeys and apes by a cat virus. *c*, Infection of the ancestors of domestic cats and jungle cats by a primate virus. Three possible times for the infection are shown and discussed in the text: (I) before the Old World monkeys and apes diverged (>30 Myr ago); (II) before the Old World monkeys diverged ($>5-10$ Myr ago); (III) recently by a baboon C-type virus (<10 Myr ago).

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letters to nature

Equivalence principle: 60 years of a misuse?

EVER since Einstein introduced the equivalence principle and used it, in conjunction with the Doppler effect, to derive the gravitational redshift of spectral lines¹, this argument has been an ingredient of many²⁻⁶ (though not all⁷⁻¹¹) expositions of relativity theory. We show here that on this point Einstein was in error, although the result found is correct (to first order).

Briefly, the usual reasoning runs that a source S emits a photon of frequency ν_s towards a detector D when both are relatively at rest at distance l apart. At the same instant the detector commences a relative motion with constant acceleration g relative to, and away from, S. D receives the photon at time $t \sim l/c$ when it has acquired a relative velocity $v = gt \sim gl/c$. The observed frequency ν_D at the detector is therefore subject to a Doppler redshift and is given to first order by

$$\nu_D = (1 - v/c)\nu_s = (1 - gl/c^2)\nu_s \quad (1)$$

The frequency of a photon is therefore redshifted as it ascends a gravitational field if, by the equivalence principle, the acceleration is replaced by a gravitational field g acting in the opposite direction, that is from D to S. Previously synchronised clocks therefore run slower the deeper they are placed in a gravitational field and hence space-time acquires a non-Minkowskian metric.

This reasoning uses the first order longitudinal Doppler effect which is a part also of Newtonian physics. Since 'fictitious' forces, and the equivalence principle, can be considered to be also part of Newtonian physics the reasoning just offered leads to a gravitational redshift and a time-transformation in Newtonian physics. This is an absurd conclusion, since Newtonian physics operates with an absolute time.

The cause of the trouble lies in the application of the equivalence principle. All physical measurements must be imagined to be made with respect to the accelerated frame, but the Doppler formulae involve two different frames (normally both inertial, although generalisations are possible). We have gone back to fundamentals and considered pairs of signals (which is how the Doppler formulae are deduced) and obtained an argument (too lengthy to be set out here) which satisfies the constraint that the gravitational redshift is zero in Newtonian physics, but satisfies equation (1) to first order if special relativity is used and g is interpreted to be a gravitational acceleration. Our argument for Newtonian physics uses a particle picture of light and attributes different velocities to the two signals.

A manuscript giving details will be submitted shortly. Here we merely observe that as a result of what has been said, it is not to be expected that the incorporation in the usual Doppler shift argument of a more accurate Doppler shift expression can be of relevance to the gravitational redshift, as recently proposed¹². This argument leads to a relationship between measurements in the inertial frame and a non-inertial frame, and is thus not suitable for a discussion of the gravitational redshift. What is achieved in ref. 12 is the following: If in the argument at the beginning of this letter D is undergoing a constant proper acceleration g away from S, then equation (1) holds exactly. This is a correct result for Doppler shifts arising in hyperbolic motion.

We note, furthermore, that in a gravitational redshift formula which is exact within the framework of special relativity and the equivalence principle (that is, for flat space-times), the way in which lengths are imagined to be measured affects the result. If l is the proper or ruler distance between the stationary source and detector in a gravitational field $g(x)$, which can be a function of distance and which is parallel to SD, then we find that equation (1) is indeed exact in the above sense, g being the gravitational acceleration at D. If, however, either coordinate distance or radar distance are used we find that equation (1) holds only to first order.

These results are clearly relevant to ref. 12 and any discussion it may precipitate. N.T.B. thanks the Science Research Council for a research studentship.

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Gravitational searchlight and its astrophysical applications

We wish to point out an interesting effect associated with light emitted in the forward direction by a source circulating in an equatorial plane around a highly collapsed object or a black hole of mass M . The essential feature of the emission is that provided the radius a of the orbit in Schwarzschild's co-ordinates exceeds $3GM/c^2$, the light emitted is blueshifted when received by a distant observer. More specifically, if ν_0 is the frequency of light at emission and ν the frequency at reception at a radial coordinate $R \gg R_s = 2GM/c^2$, we have

$$\frac{\nu}{\nu_0} = \frac{\sqrt{[(2-3\xi)(1-\xi)]}}{\sqrt{[2(1-\xi)]} - \sqrt{\xi}} \quad (1)$$

where

$$\xi = R_s/a \quad (2)$$

While $\nu > \nu_0$ for all values of $\xi < 2/3$, it can be shown easily that as $\xi \rightarrow 2/3$, the blueshift diverges. Writing $\xi = (2/3)(1-\epsilon)$,

$$\nu \simeq (2/3) \nu_0 \epsilon^{-1/2} \text{ as } \epsilon \rightarrow 0 \quad (3)$$

Thus very high blueshifts are obtained as the orbit of the particle tends to the so called unstable circular orbit. Qualitatively, we can understand the result in terms of the competition between the Doppler blueshift of forward light emission and the gravitational redshift due to the central mass. The former increases much more rapidly than the latter as $\epsilon \rightarrow 0$. We are interested here with orbits with very small ϵ , that is, orbits close to the unstable circular orbit.

The behaviour of matter revolving very close to such an orbit has been considered by several authors¹⁻³. For example, an electron moving in a circular orbit can emit synchrotron-type radiation. It is also possible for gravitational radiation to be emitted by revolving matter. Such effects, however, have turned out to be very small even in the limiting case of $\epsilon \rightarrow 0$. Here we suggest an alternative situation where the above mentioned result can play a significant part.

Before coming to the astrophysical setting consider what happens when we have matter moving in a thin ring round an object with mass M , with say $\epsilon_1 \leq \epsilon \leq \epsilon_2 \ll 1$. Suppose matter in this region has uniform distribution and is emitting light in a band of frequencies peaked round ν_0 , say. Then purely geometrical considerations and equation (3) lead to a reception spectrum at R of the form

$$F(\nu) = A(\nu_0) \nu^{-1}, \nu \geq \nu_0 \quad (4)$$

The function $A(\nu_0)$ depends on the dimensions of the object and the emissivity of matter under consideration. The derivation of equations (3) and (4) is somewhat involved and will be published elsewhere, but the ν^{-1} dependence of the spectrum purely on geometrical ground is noteworthy.

Turning now to the astrophysical considerations, it is well known that a highly collapsed object or a black hole tends to accrete matter. In general the infalling matter has some angular momentum so that it does not fall in radially. Instead, one can imagine matter making revolutions round the object several times before falling in. The unstable circular orbit is likely to play an interesting part in this process. The infalling matter goes round in orbits with small ϵ several times before getting

sucked in or thrown out again¹. So we may consider the thin ring discussed above as made up of transient matter.

The ν^{-1} spectrum is common to many extragalactic sources of radiation. We have considered the continuum emission of QSOs whose spectrum can be approximated this way⁴. By considering thermal emission from hot gas in a narrow range of frequencies around $\nu_0 \sim 3 \times 10^{14}$ Hz and taking the emissivity⁵ as $\sim 4.4 \times 10^{-24} n_e^2 \text{ erg cm}^{-3} \text{ s}^{-1}$, with n_e the electron density in cm^{-3} we find that in order to explain the observations of a QSO like 3C273 in the optical and ultraviolet region on the basis of equation (4) we need a mass-distance relationship of the form

$$M/M_\odot \simeq 1.7 \times 10^{15} (R_{\text{mpc}}/n_e)^{2/3} \quad (5)$$

Here R_{mpc} is R expressed in Mpc. In a 'local hypothesis', R_{mpc} may not exceed ~ 100 while n_e can be taken as high as $\sim 10^9$ to give a mass M of the order of that of a galaxy. Provided thermal emission is confined to relatively narrow band of frequencies at the source, the resulting spectrum at a large distance will show a ν^{-1} dependence.

The light emitted in the forward direction makes several rounds of the object before reaching the distant receiver. As $\epsilon \rightarrow 0$, the angle through which the light has turned can be shown to be

$$\phi \sim \ln[(24 - 12\sqrt{3})/\epsilon] \quad (6)$$

Thus for $\epsilon \sim 10^{-10}$ the light will go round nearly four times.

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Solar energetic particle event with ${}^3\text{He}/{}^4\text{He} > 1$

OBSERVATIONS of γ rays and the short lived isotope ${}^3\text{H}$ provide evidence for the occurrence of nuclear reactions by high energy particles accelerated in solar flares^{1,2}. Stable isotopes such as ${}^2\text{H}$ and ${}^3\text{He}$ should also be produced in these reactions. Solar ${}^3\text{He}$ particles were first detected by Hsieh and Simpson³. In the energy range 10 to 100 MeV per nucleon they obtained ${}^3\text{He}/{}^4\text{He} = (2.1 \pm 0.4) \times 10^{-2}$ by summing over seven solar particle events. Garrard *et al.*⁴ and Anglin *et al.*⁵ have reported that ${}^3\text{He}/{}^4\text{He}$ was highly variable from event to event. (Table 1). In the ' ${}^3\text{He}$ -rich events', ${}^2\text{H}$ and ${}^3\text{H}$ were not detected and the resulting upper limits were much less than expected from the theory of nuclear reactions⁶. These events were small, and the

Table 1 Relative abundance of He isotopes

$^3\text{He}/^4\text{He}$ Solar particle events	$^4\text{He}/^1\text{H}$ Solar particle events	$^3\text{He}/^4\text{H}$ Solar particle events
1.52 ± 0.1 , May 28, 1969 (this paper)	0.40 ± 0.04 May 28, 1969	0.60 ± 0.05 , May 28, 1969
0.54 ± 0.09 , July 30, 1970 (ref. 15)	$\sim 10^{-2}$, average of seven flares in 1967 (ref. 3)	~ 0.09 , July 30, 1970
0.26 ± 0.08 , October 14, 1969 (ref. 4)	$\sim 1.7 \times 10^{-2}$ (ref. 16, average of seven large flares in solar cycle 20)	~ 0.005 , October 14, 1969
0.077 ± 0.02 , November 2, 1969 (ref. 8)		~ 0.0005 , general range of occurrence in ordinary solar events
0.021 ± 0.004 , average of seven flares in 1967 (ref. 3)		
Chromosphere $\leq (1.0 \pm 0.5) \times 10^{-2}$ (ref. 9)	$\sim 1.4 \times 10^{-4}$, September 25, 1969 (Dietrich and Simpson, unpublished)	
Solar wind 4×10^{-4} (ref. 11)	Chromosphere and prominences ~ 0.06 (ref. 12)	
$\leq 4 \times 10^{-4}$ (ref. 10)	Solar wind ~ 0.045 (ref. 17)	

number of ^3He particles observed was low (~ 70). This anomalous production of ^3He should provide new insight into the acceleration and confinement process of energetic particles in solar flares.

Using the Goddard cosmic-ray telescopes of OGO-V (ref. 7) we detected an unusual solar event on May 28, 1969. The flare associated with the event occurred at 1248 local time and had importance 1B. The solar coordinates of the event are 10°N and 56°W with the associated McMath plage region 10109. About 600 ^3He nuclei were detected in this event and $^3\text{He}/^4\text{He} = 1.52 \pm 0.1$ in the energy range 4 to 80 meV per nucleon. This is the highest ratio reported so far for any solar event.

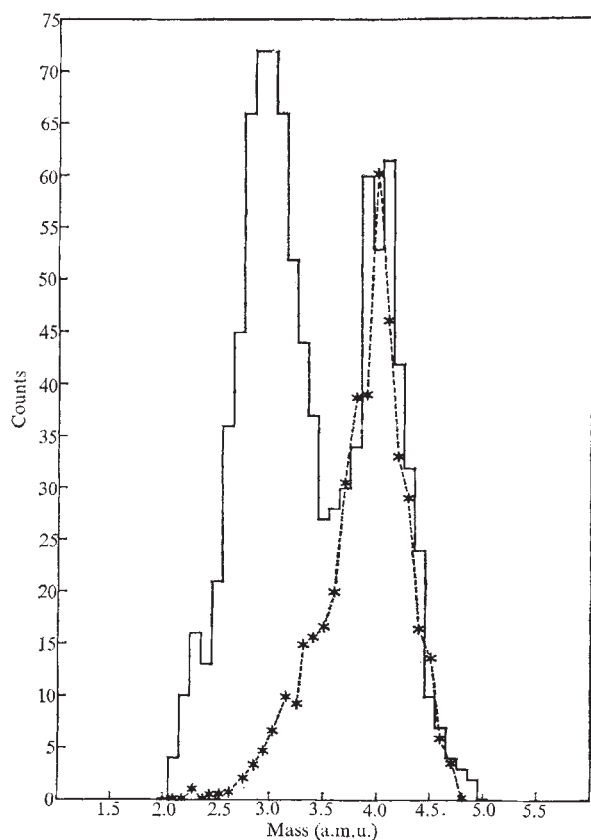


Fig. 1 Mass histogram for He nuclei observed in the solar particle events on May 28 and November 2, 1969. Note dominance of ^3He in the May 28 event. The $^3\text{He}/^4\text{He}$ ratio for the November 2 event is 0.08, in agreement with that reported by Dietrich⁸. —, May 28 event; *, November 2 event.

In Fig. 1 we give the mass histograms for He nuclei. The dotted curve represents the mass distribution (normalised at the peak of ^4He distribution) for another event on November 2, 1969. For this event we get $^3\text{He}/^4\text{He} = 0.08$, in excellent agreement with Dietrich⁸.

The ratio of hydrogen to helium for the event on May 28 is also highly unusual with $^1\text{H}/(^3\text{He} + ^4\text{He}) = 1.00 \pm 0.05$ (Table 1).

The energy spectra of three components were all consistent with power laws in kinetic energy per nucleon. The exponents for ^1H , ^3He and ^4He were -4.29 ± 0.10 , -4.16 ± 0.10 and -4.04 ± 0.11 , respectively.

The observed upper limit to $^3\text{He}/^4\text{He}$ in the solar chromosphere was $(1 \pm 0.5) \times 10^{-2}$ (ref. 9). In the solar wind $^3\text{He}/^4\text{He} \sim 10^{-4}$ (refs 10 and 11). The solar particles have higher relative abundance of ^3He in comparison with the solar chromosphere. The $^4\text{He}/^1\text{H}$ ratio is also large compared to the observations on solar prominences¹². In Table 1, the isotopic abundances of He as obtained from several related studies are shown for comparison.

Ramaty and Kozlovsky¹³ have proposed a theory dealing with the production of ^2H , ^3H and ^3He in solar events. They have taken into account effects of kinematics of the reactions and the possible anisotropy of solar beams to explain the anomalously small ^2H and ^3H abundances compared to ^3He . They find that for energetic protons and ^4He nuclei incident on the solar atmosphere, ^3He will be preferentially emitted in the direction opposite to that of the incident particle whereas ^2H and ^3H will tend to have the same direction. For the giant flare in August, 1972 (ref. 14), they arrive at about 2 gm cm^{-2} to account for $^3\text{He}/^4\text{He}$ of ~ 0.02 observed in the event. They also relate the γ -ray intensities observed by Chupp *et al.*⁷ with the energetic protons observed in the event. The large production of ^3He according to Ramaty and Kozlovsky, occurs only at low energies (about 0.1 meV per nucleon) and so some post acceleration is necessary to account for the observed particles.

Using the same model to explain the very high $^3\text{He}/^4\text{He}$ ratio in the May 28 event requires the passage through 2.3 nucleon mean free paths or some 170 g cm^{-2} of material for protons. This further requires that the ionisation energy loss be balanced by continuous acceleration which means that a 50 MeV per nucleon He nucleus would have to receive a total energy of $\sim 12 \text{ GeV}$. This seems highly implausible. One possible clue may be provided by the simultaneous observation of the solar X rays and electrons. Here it is found that only 10^{-2} to 10^{-3} of the electrons escape with a large fraction incident on the lower corona which produces the X-ray emission. A similar effect may be occurring with the nuclear component though the rigidities of the nuclear particles are larger by factors $> 10^2$. The other secondary products ^2H and ^3H are not seen in this event. Assuming that one nucleus of ^2H (or ^3H) was seen during the event, the upper limit for $^2\text{H}/^3\text{He}$ (or $^3\text{H}/^3\text{He}$)

is $\sim 1/600$. Simultaneous γ -ray observations would be of great value in identifying the type of process and the time scale on which it operates. These were not available for this event. It is also possible that the solar material involved in these flares has an anomalous composition to start with.

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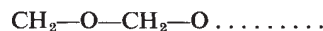
Formaldehyde polymers in interstellar space

THE precise composition of interstellar dust is as yet unknown. Recent studies of the wavelength dependence of extinction and polarisation favour a composite model of three components: (1) elongated dielectric particles of radii $\sim 10^{-5}$ cm, which would explain the data in the 1–0.3 μ m spectral region; (2) almost spherical graphite particles of radii $\leq 2 \times 10^{-6}$ cm, which would explain the near and middle ultraviolet data in $0.3 \gtrsim \lambda \gtrsim 0.2 \mu$ m; and (3) small dielectric particles which contribute mainly to extinction and albedo at wavelengths, $\lambda \lesssim 0.2 \mu$ m.

There is considerable evidence to support the view that silicate grains exist in circumstellar envelopes around certain cool stars¹. An emission feature in the 8–12 μ m waveband occurring in the spectra of many cool Mira-type stars has been identified with particles of a mineral-like composition. It is also widely believed that silicate dust is responsible for a major part of the observed interstellar extinction and polarisation at visual wavelengths, in other words, that silicates make up component (1) above. The evidence for this is not convincing and several strong counter indications already exist. Applications of the Kramers–Kronig relationship to the observed interstellar extinction curve suggest that greater relative abundances

of Mg, Si to H than are consistent with cosmic abundance ratios are necessary to explain the observed extinction^{2,3}. This discrepancy could be as much as a factor 10. It seems therefore, that the only elements present in sufficient quantity in the interstellar medium to contribute to the observed visual extinction coefficient, $\kappa_v \sim 2 \text{ mag kpc}^{-1}$, are C, O and N. The possibility of O in the form of H₂O ice has been discussed for many years, although a 3.1 μ m ice band was not detected in the spectra of several moderately reddened stars^{4,5}.

I have argued (N.C.W., unpublished) that formaldehyde molecules which have been detected in over a hundred interstellar clouds could condense on silicate grains. Condensation of H₂CO on silicate grains with temperatures $\lesssim 20$ K is attended by polymerisation into chains



resulting in the formation of a crystalline polymer known as polyoxymethylene (POM). The constituent elements of this material are sufficiently abundant to provide the main contribution to the mass density of interstellar dust. These particles would grow as long whiskers under interstellar conditions and possess the required dielectric properties to account for component (1) in the composite grain model. In common with many other polymers, POM is moderately refractory with a melting temperature in the range 450–500 K. Such grains could survive in H II regions and radiate at temperatures $T_g \lesssim 500$ K thus accounting for many galactic infrared sources.

The optical refractive index of epoxy formaldehyde resin, which is likely to be similar to that of POM, is $n = 1.58$ (ref. 6). A cylindrical particle of radius close to 1.5×10^{-5} cm, with this value of n , possesses extinction and polarisation properties which are consistent with interstellar data⁷.

The strongest evidence adduced in support of the view that silicate grains are mainly responsible for the general interstellar extinction is the appearance of an 8–12 μ m emission feature in the infrared spectrum of the Trapezium nebula, as well as a similar extinction feature in the spectrum of the BN star in the Orion nebula^{1,8,9}. Most other 10 μ m features observed in stars are confined to strictly circumstellar grains and do not necessarily relate to the behaviour of interstellar grains.

By a remarkable coincidence, POM polymers also exhibit strong optical activity in the 8–12 μ m waveband. Figure 1 shows the absorption spectrum of POM films studied by Tadokoro *et al.*¹⁰ for polarised light. Although this feature is comprised of two main bands, as in the case of silicates, a distribution function in average chain length will mask much of the fine structure, as the precise positions of individual bands depend on this parameter in the crystalline powder.

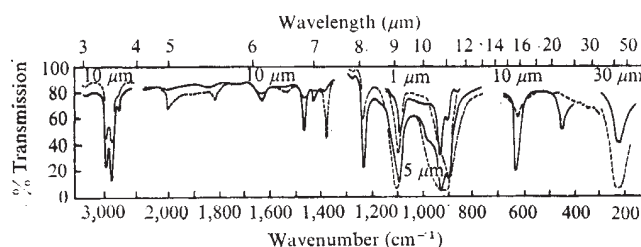


Fig. 1 Infrared transmission spectra for polarised light of POM-films of several thicknesses (1, 5, 10 and 30 μ m) for the various wavelength intervals as indicated in the insets. The solid curve refers to light with electric vector perpendicular to crystal axes; dashed curves for electric vector parallel to the crystal axes. (Differing thicknesses of film, 1, 5, 10 and 30 μ m, are used over various wavelength intervals. When this is noted, the 8–12 μ m bands are considerably stronger than others indicated in these data.)

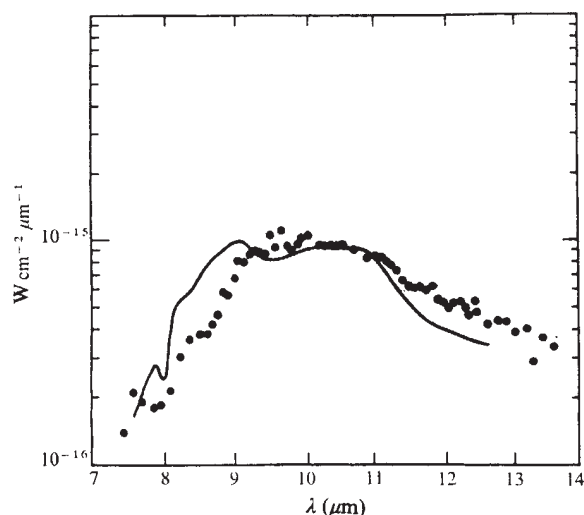


Fig. 2 The infrared emission (ω) from the Trapezium nebula compared with the theoretical flux from 445 K POM polymer grains (solid curve).

The average mass extinction coefficient at the centre of this band, estimated from the data of Tadokuro *et al.*¹⁰, is

$$\kappa_{10\mu m} \simeq 1.4 \times 10^3 \text{ cm}^2 \text{ g}^{-1} \quad (1)$$

assuming a specific gravity $s = 1.4$ for POM. At the visual wavelength, $\lambda_v = 5,470 \text{ \AA}$, the mass extinction coefficient for POM grains of radii, $a = 1.5 \times 10^{-5} \text{ cm}$, is

$$\kappa_v \simeq (3Q_{\text{ext}}/4as) \simeq 7.1 \times 10^4 \text{ cm}^2 \text{ g}^{-1} \quad (2)$$

Equations (1) and (2) give $\kappa_v/\kappa_{10\mu m} \simeq 51$. The corresponding ratio inferred from the astronomical data for extinction in the BN star is very similar ($\kappa_v/\kappa_{10\mu m})_{\text{BN}} \simeq 50$ (ref. 1). Figure 1 shows that interstellar POM grains could give rise to somewhat weaker, yet probably detectable, features at $\lambda \simeq 3 \mu\text{m}$ and $\lambda \simeq 16\text{--}23 \mu\text{m}$. The latter feature imparts a further ambiguity to the identification of silicate grains, whilst the former may be confused with the $3.1 \mu\text{m}$ band of H_2O ice.

Absorbance data for heated POM films¹⁰ may be used to compute an approximate emission spectrum of small POM grains. The absorption efficiency of small particles, irrespective of shape, is proportional to the absorbance γ_{abs} , provided the refractive index n is close to 1 and the absorptive index k remains small throughout the band. Assuming these conditions to be fulfilled the emission spectrum of POM grains may be calculated by

$$F_\lambda \propto \gamma_{\text{abs}} B_\lambda(T_g)$$

where $B_\lambda(T_g)$ is the Planck function, T_g is the grain temperature. The emission spectrum of the Trapezium nebula is shown in Fig. 2 together with a normalised emission spectrum for POM grains heated to 445 K. The agreement is as good as, if not better than, for any silicate model. POM grains are thus able to explain all available interstellar extinction and emission data.

In view of the spectral identifications presented here POM grains must clearly be regarded as a strong candidate for the main component of interstellar dust. Such grains could contribute a significantly higher mass density than silicate grains. More detailed measurements of the complex refractive index of POM as a function of wavelength and of temperature are required before further comparisons with astronomical data can be made.

Our provisional conclusion is that interstellar grains consist of a mixture of small graphite particles and silicate particles coated with POM mantles. The graphite and silicate particles

are ejected from cool giant stars; silicate grains have low enough temperatures under interstellar conditions to accrete POM polymer mantles.

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Pacific equatorial pressure gradient and Indian monsoon rainfall

SEA surface temperature (SST) anomalies are known to affect atmospheric flow patterns considerably. The Indian monsoon circulation is induced by the thermal contrast between the Asian continent and the Pacific and Indian oceans and by the orography of the Himalayas, so large scale SST anomalies are likely to affect the monsoon circulation, but after some time lag.

In recent years SST anomalies over the Pacific have been associated with Walker's Southern Oscillation^{1,2}. We find that the factor (Mataveri-Darwin) pressure difference used by Quinn and Burt as a measure of the strength of the Southern Oscillation is also quite useful in predicting monsoon rainfall.

Figure 1 shows the relationship between the seasonal (June-September) monsoon rainfall of north-west India and the mean

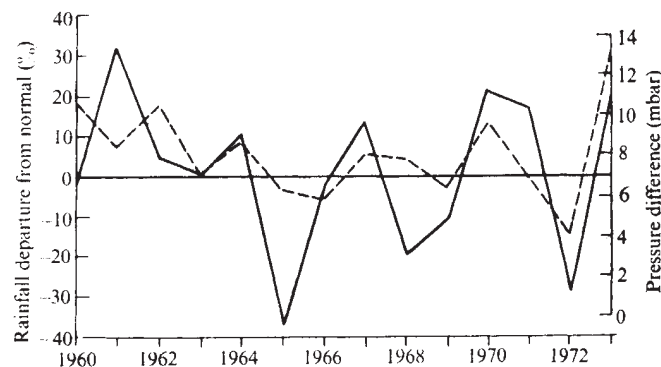


Fig. 1 Relationship between rainfall and pressure gradient. —, Departure from normal (%) for seasonal (June-September) rainfall in north-west India. — —, Mean Pacific pressure gradient (Mataveri-Darwin) during April and May.

Pacific pressure gradient (Mataverí-Darwin) during April and May, from 1960 to 1973. There is some correspondence between the two curves (except for 1968) and the large contrast between the drought year of 1972 and the good monsoon year of 1973 is evident in both cases. In the drought years of 1972, 1965 and 1966, this average pressure difference fell below 7 mbar. The area of that part of the pressure gradient curve below 8 mbar, as used by Quinn and Burt, seems to be a good indicator. In 1972, an El Niño year, the pressure difference fell to as low as 0.5 mbar in May, whereas it was 7.6 mbar in May 1973. Pacific typhoon activity was also much above normal in 1972 but below normal in 1973.

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New pole for early opening of South Atlantic

USING the trends of equatorial, marginal fracture ridges, Le Pichon and Hayes¹ proposed an early phase for the opening of the South Atlantic, with a pole of rotation at 21.5°N, 14°W with respect to Africa. Francheteau and Le Pichon² tested this plate tectonic model with the whole South Atlantic and assumed that there is a relationship between continental margin offsets, the subsidence of coastal basins, and adjacent marginal fracture zones. We have studied extensions of fracture zones

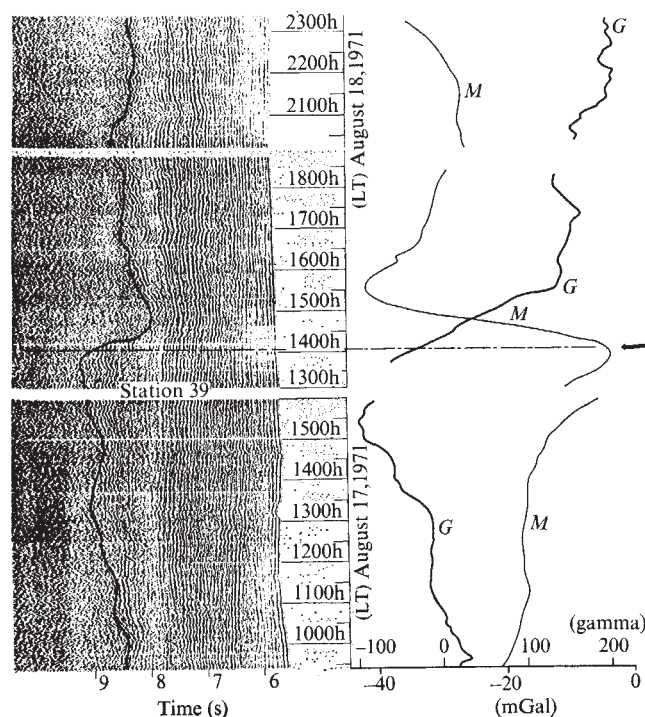


Fig. 1 Seismic profile across the Chain Fracture Zone south of Togo (location on Fig. 2). Note the difference in level of the acoustical basement under a thick sedimentary cover. The free air gravity anomaly is of the order of 40 mGal. The arrow indicates the theoretical location used for the determination of the trend of the fracture zone. M, Magnetic curve; G, gravity curve.

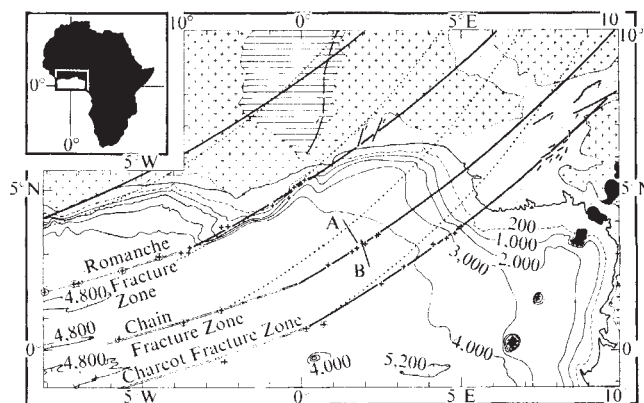


Fig. 2 Eastern Gulf of Guinea. Bathymetric contours (m) from E. Uchupi (unpublished); geological sketch from the geological map of Africa¹⁹. Crosses, cratonic area; hatched, Palaeozoic platform; dotted, Mesozoic to Recent basins; black area, Cameroon volcanic line. Heavy crosses, positions of geophysical features (from seismic, gravity, or magnetic surveys) used for computation of the new early pole of opening. Heavy black lines, portion of small circles about the new early pole along the Saint-Paul, Romanche, Chain and Charcot Fracture zones. Note the good agreement between the extension of the two last fracture zones and the main geological trends in Nigeria. Dotted lines, portions of small circles about the previously proposed pole of opening¹. Note the discrepancies with the geological trends in the Benoue Trough area. Double lines, posterior fracture zone trends. A-B indicates profile of Fig. 1.

in the Gulf of Guinea and adjacent continental margins and have determined a new early opening pole that differs markedly from the one previously determined.

Thanks to deeper penetration seismic techniques, extensions of the well known equatorial fracture zones³ have been traced into the Gulf of Guinea⁴⁻⁷ under a thick sedimentary cover mostly related to the Niger Delta. Figure 1 shows a seismic section across the extension of the Chain Fracture zone. It has been proposed⁸ that the position of the inflexion point on the regional free-air anomaly curve corresponds to the main changes in the average basement level; consequently, seismic and gravity criteria have been used both together and separately to locate the position of the fracture zone. A structural trend called the Charcot Fracture Zone^{7,9,10} has been discovered just south-west of this delta (Fig. 1).

We have re-interpreted these results to obtain a better knowledge of the early opening phase of the Gulf of Guinea and consequently of the South Atlantic. The positions determined of the main geophysical features considered here are shown on Fig. 1. The Saint Paul Fracture Zone itself has not been taken into account, mainly because of its complex structural pattern near the Liberian continental margin¹¹. The trend of the Chain Fracture Zone previously inferred from bathymetric data^{1,5} is not compatible with our data (Figs 1 and 2). Parameters of our new pole are 32°N, 20°W (with a standard deviation of 6 km).

The great circle defined by the two poles of rotation (21.5°N, 14°W and 32°N, 20°W) cuts across the Liberian continental margin, the Walvis Ridge and the Agulhas Fracture Zone^{12,13} along a line close to the boundary that existed between Africa and South America before the opening of the ocean. Consequently, small circles about the two poles of rotation are tangential to each other, especially in the area where the great circle lies close to preopening boundaries. They diverge as the distance from the great circle increases, as in the eastern part of the Gulf of Guinea. Moreover, this effect decreases as the distance from the pole increases.

Figure 2 shows the small circles about each pole of rotation in the Gulf of Guinea. The direction of early opening which we propose fits well with the Liberian-Ivory Coast continental slope and with the limit of the Togo-Dahomey Basin. It also

agrees much better than did the previously proposed direction with the broad geological structure in Nigeria. It allows the extension of the Chain Fracture Zone to be related more easily with the northern hinge line of the Niger Delta¹⁴ and the northern limit of the Benoue Trough¹⁵. The Charcot Fracture Zone seems to correspond well with the complex Abakiliki area in southern Nigeria¹⁶.

Figure 3a shows the good agreement between small circles about the new pole (32°N, 20°W), and fracture trends in the vicinity of the Liberian continental margin¹¹. Figure 3b and c represent, respectively, the easternmost section of the Walvis Ridge¹⁷ and the Agulhas Fracture Zone^{12,13,18} on which a computed theoretical direction from the new pole has been superimposed. As expected from the orientation of the great

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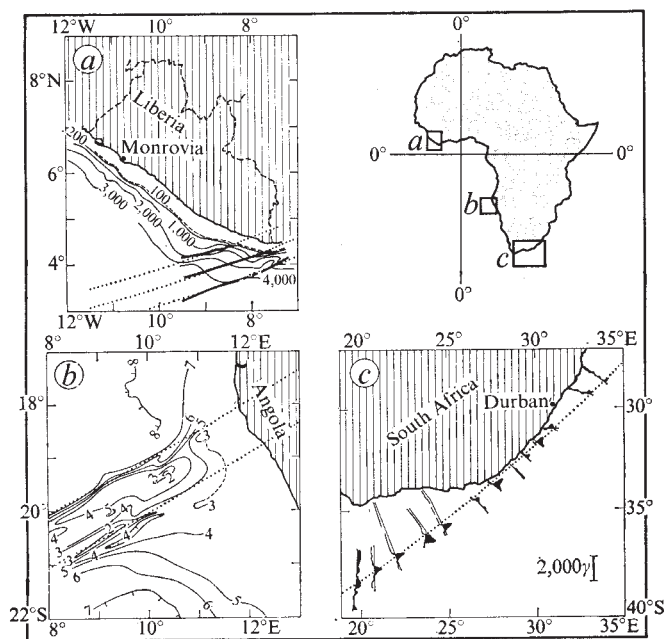


Fig. 3 Comparison between some western South Atlantic marginal structures and the new theoretical direction (small circles about a pole at 32°N, 20°W). *a*, Liberian continental margins (depth contours in metres) and the Saint-Paul, Cape Palmas, Grand Cass fracture zones¹⁰; *b*, depth (in seconds) of easternmost Walvis Ridge basement⁶; magnetic anomalies in the Agulhas Fracture Zone.

circle and the distance from the poles, the agreement in both cases is good. It is interesting to note that the easternmost Agulhas Fracture Zone differs somewhat from the theoretical direction but it should be specified that this fracture zone has for the most part been inferred from magnetic anomalies¹³. Those anomalies could be related partly to the continental-oceanic crust boundary instead of being representative of only the fracture zone itself.

Although in many cases this newly established evidence will have to be taken into account in any detailed reconstruction of the opening of the Atlantic, the conclusions of Francheteau and Le Pichon² still seem to be valid.

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Magnetic polarity of Explorer Ridge basalts

THE Vine–Matthews hypothesis¹ connects geomagnetic polarity reversals with marine magnetic anomalies through the process of seafloor spreading. This hypothesis requires a one-to-one correlation between the sign of a given marine magnetic anomaly and the magnetic polarity of underlying oceanic basalt. Because properly oriented samples are lacking, however, this feature remains virtually untested in spite of its crucial significance.

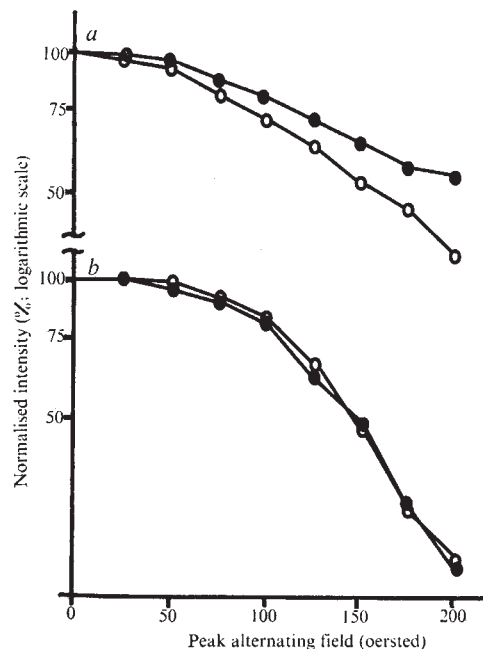


Fig. 1 Demagnetisation curves interpreted as: *a*, reversed polarity (specimen 717002A); *b*, normal polarity (specimen 701520A). The size of the symbols represents experimental uncertainty. ●, NRM; ○, ARM acquired in a bias field of 0.58 oersted and a peak alternating field of 1,800 oersted.

Table 1 Overhead magnetic anomalies and inferred polarities

Dredge site	Location*	Overhead anomaly†	Age (Myr)‡	Specimen	Δ_0	Δ_{25}	Δ_{50}	Δ_{75}	Δ_{100} §	Inferred polarity
7004	50° 14.1'N 130° 15.4'W	+200 γ	<1	700407A	+1.3	+0.4	-0.8	-0.5	-1.3	N
				700418A	+2.6	-0.6	-0.4	¶	-0.1	N
				700425A	+4.5	+4.3	+1.8	+2.4	+2.5	(N?)
				700427A	+3.0	+1.9	+1.9	+1.3	+2.7	N
				700434A	+1.5	+1.7	+1.5	+1.3	+0.3	N
				700465A	+6.1	+7.0	+7.6	+8.2	+8.0	R
7015	49° 45.3'N 130° 17.3'W	+300 γ to +400 γ	<1	700482A	+1.3	¶	-1.5	-2.8	-1.2	N
				701504A	+0.9	-3.0	-2.6	+1.0	+0.6	N
				701520A	-0.6	-1.2	-0.3	-0.7	-0.1	N
				701522A	-1.3	-1.2	+0.6	-0.8	-0.2	N
				701523A	-2.0	-1.8	-2.2	-0.4	+0.7	N
				701525A	-0.7	-1.9	-2.3	+1.1	-2.3	N
				701533A	-1.1	-1.0	-1.0	-1.3	-1.2	N
				701534A	+0.5	-1.2	-1.0	-0.8	-1.0	N
7017	50° 03.8'N 129° 41.8'W	+300 γ to +600 γ	<1	701701A	+0.4	-0.1	-0.9	+5.3	+5.2	(R?)
7170	49° 07.9'N 130° 34.8'W	-300 γ	1 to 2	717001A	+7.3	+7.6	+7.4	+7.3	+9.1	R
				717002A	+9.0	+9.1	+9.6	+8.5	+7.4	R
				717004A	+8.0	+8.1	+7.8	+6.8	+5.9	R
				717008A	+6.4	+5.7	+6.8	+7.2	+9.8	R
				717009A	+8.0	+7.1	+7.5	+7.4	+6.3	R
7192	50° 12.1'N 129° 52.6'W	-500 γ	1 to 2	719202A	+5.4	+2.3	+6.3	+10.2	+12.1	R
				719203A	+7.6	+2.9	+4.3	+3.4	+13.1	R
				719204A	+4.3	+6.9	+13.3	+19.7	+11.6	R
				719207A	0	-1.9	-0.5	-4.3	¶	N
				719208A	+0.6	-1.5	-1.0	-3.6	-0.8	N
				719210A	+0.8	-0.1	+2.1	+3.7	+8.5	(R?)

* Best satellite Navigator fix for terminus of dredge haul.

† From data of Raff and Mason¹³; range given where hauls crossed steep gradients.

‡ From interpretations of magnetic anomalies by Vine⁹ and Silver¹⁰.

§ $\Delta h = \frac{100}{N} \sum \delta_i$, where h is the normalising field; N is the number of demagnetising steps between h and 200 oersted, and $\delta_i =$ (normalised NRM—normalised ARM) at field i .

¶ Demagnetising step inadvertently omitted.

Three cases, discussed in the text, have tentative polarity assignments only and are designated by parentheses.

Under certain circumstances^{2,3}, the polarity of the original remanence of unoriented dredged samples can be recovered. It is essential for the success of the method that there be present secondary magnetic components in the samples resulting from magnetic viscosity of the oceanic basalts in the presence of the recent geomagnetic field, and these can be expected to parallel the primary remanence of normal polarity basalts and to oppose that of reversed material, provided that the rocks have remained *in situ*. Under appropriate conditions this may lead to a detectable difference between the alternating field demagnetisation curves of natural remanence (NRM) and laboratory induced remanence; anhysteretic remanence (ARM) is used to avoid problems associated with reheating. Irving² has suggested that a normalised NRM curve lying significantly above the corresponding ARM curve is indicative of reversed primary remanence.

This method (and slight variations of it) has been applied to samples from the Mid-Atlantic Ridge. Agreement has been found^{4,5} between inferred polarity and overhead anomaly in 20 out of 41 specimens. A positive correlation has been found in 16 out of 20 specimens dredged from the Juan de Fuca Ridge⁶.

We are completing a study of the magnetic properties of a suite of basalt samples dredged from the Explorer Ridge which represents an extension of the Juan de Fuca Ridge north of the Sovanco Fracture Zone^{7,8}. The details will appear elsewhere; here, we report a substantial correspondence between inferred magnetic polarities and those predicted by the Vine-Matthews hypothesis. Twenty-six basalt samples (one specimen per sample) from five separate dredge hauls have been investigated. The locations of the five hauls (Table 1) lie within the central positive magnetic anomaly and the adjacent negative anomaly^{9,10}. They are within 40 km of active spreading centres and range in age from 0–2 Myr.

We have found that the region between 0 and 200 oersted is

the most useful part of the coercivity spectrum as the ARMs become weak and generally unstable at higher peak fields. Results indicating clear cases of each polarity are given as examples (Fig. 1). The 26 specimens have been classified according to the relative hardness of their ARM and NRM demagnetisation curves.

One must seek assurance that any apparent differences between NRM and ARM coercivity spectra are significant compared with experimental uncertainty. For strongly magnetised rocks, this is no better than about 2%. To be conservative we have sought an average difference of 4% to establish firmly any divergence of the two curves, although Table 1 indicates that this choice is not too critical for most of the samples. In addition, the alternating field (a.f.) treatment to which the two curves should be normalised must be investigated fully. This second point arises because of the magnetic noise generated by additional short-lived components of magnetisation acquired between departure from outcrop and measurement². These unwanted viscous components can be effectively filtered off by normalising to successively higher fields³. To test the difference, or lack of difference between NRM and ARM, and also to investigate the effect of different normalising fields, the mean difference, Δ_h (ref. 5) between the two spectra at the various treatments has been calculated. The values of Δ at different normalising fields, h , are given in Table 1. As the appropriate normalising field is not known in advance we first of all classified those cases in which all normalisations yielded the same inferred polarity. Thus, 14 specimens could be regarded as definitely normal and 7 specimens as definitely reversed. A further two specimens (719202A and 719203A) showed generally large positive differences with isolated dips below +4%; these are very probably reversed. Of the remaining three specimens, 700425A is classified as normal as its Δ values exceeds +4% (by very small margins) only at 0 and 25 oersted. Specimens

701701A and 719210A are tentatively classified as reversed because their demagnetisation curves begin to diverge significantly at higher fields.

We therefore feel that 23 of the 26 assigned polarities are quite firmly established; the remaining three are rather tentative. The significant result is that a total of 20 out of the 23 specimens (or 22 out of 26 if the dubious assignments are included) yield polarities in agreement with the overhead anomaly (Table 1), lending strong support to the Vine-Matthews hypothesis. The few cases of disagreement give no cause for alarm. Indeed, a small percentage of samples of opposite polarity are expected from gravity-transported boulders and from contamination because of the finite width of intrusive activity at accreting plate boundaries^{11,12}.

We thank the officers and crews of CFAV Endeavor and CSS Parizeau, and R. L. Chase, who directed the cruises. We also thank A. B. Reid, D. K. Bingham, D. I. Gough and J. A. Jacobs for assistance and advice. Financial assistance was provided by the National Research Council of Canada.

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3,800-Myr granitic gneiss in south-western Minnesota

We have previously arrived at an age of 3,550 Myr for granitic gneiss in the vicinities of Morton and Montevideo in the Minnesota River valley, south-western Minnesota. We now report new Rb-Sr analyses (Table 1) and an age of 3,800 Myr for the fine grained foliated phase of the Montevideo Gneiss of Lund². The rocks have undergone a complex history of metamorphism which remains to be deciphered, but the age determinations reveal that the geological mapping and previous interpretations did not provide a proper basis for sampling.

Table 1 Analytical data

Sample no.	p.p.m. (weight)		Ratio (atomic)	
	Rb	Sr	⁸⁷ Rb/ ⁸⁶ Sr	⁸⁷ Sr/ ⁸⁶ Sr
KA-209	79.4	204.1	1.132	0.7619
KA-54	75.0	270.1	0.8066	0.7455
605	80.0	287.8	0.8076	0.7434
606	89.1	256.8	1.009	0.7544
607	41.5	378.7	0.3183	0.7206
608	92.7	307.2	0.8769	0.7472
609	71.7	339.0	0.6136	0.7324

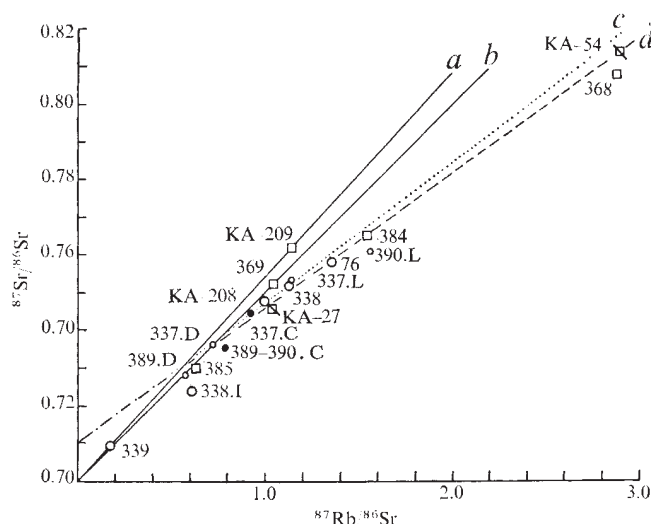


Fig. 1 Rb-Sr isochron diagram of whole-rock (squares) and K-feldspar samples (crossed squares) from the Montevideo Gneiss and whole-rock samples (large open circles) from the Morton Gneiss (small open circles, samples in composites; small closed circles, composites). (From Goldich *et al.*) a, 3,800 Myr (0.700); b, 3,550 Myr; c, 2,650 Myr (0.710); d, 2,550 Myr.

Our original age of 3,550 Myr. ($t_1 = 50 \times 10^9$ yr) was based on our interpretation of discordant U-Pb zircon ages and a Rb-Sr age computed for three whole-rock samples (Fig. 1, numbers KA-209, 369, 385). The 2,650-Myr isochron (Fig. 1) represents a time of high grade (granulite facies) metamorphism and is based on Rb-Sr rock-mineral isochrons and on nearly concordant U-Pb zircon ages¹. The 2,550-Myr isochron approximates the time of uncovering and of stabilisation of the Rb-Sr system in the K-feldspar. A line through the points for sample KA-209 from the Montevideo Gneiss and sample 339 from the Morton Gneiss gives an age of 3,800 Myr with an initial ⁸⁷Sr/⁸⁶Sr of 0.700 (Fig. 1).

We did not previously¹ attach any significance to this line because only two data points were involved. Of the six new samples from the Montevideo Gneiss, however, four lie on the 3,800-Myr isochron and two above this line (Fig. 2). The difference between the measured ratios for samples 607 and KA-54 and the other ratios are real and outside analytical error ($\pm 1\%$ for ⁸⁷Rb/⁸⁶Sr and ± 0.0002 for ⁸⁷Sr/⁸⁶Sr); hence, inclusion of these samples in an isochron plot is not desirable and some sort of geological explanation is required. The most obvious one is that during the complex history of metamorphism and igneous activity some metasomatic action was involved; for example, Rb and Sr moved among the rock units creating an open system. A gain in Rb or loss in radiogenic ⁸⁷Sr would lower the ages and explain the scatter of the whole-rock and K-feldspar points in Fig. 1. The apparent ages of the gneiss samples (Fig. 2) therefore must be considered minimum values. If it is argued that the apparent ages are too old, the explanation must invoke either loss of Rb or gain of radiogenic ⁸⁷Sr. We favour such an explanation with a net gain of ⁸⁷Sr for samples KA-54 and 607.

A least squares calculation, excluding KA-54, 607 and 339, gives an age of $3,950 \pm 70$ Myr (2σ) with an initial ⁸⁷Sr/⁸⁶Sr ratio of 0.698 ± 0.004 , exceptionally low for terrestrial rocks. We, therefore, prefer to include sample 339 from the Morton Gneiss as a guide to the initial ratio of 0.700 and an age of 3,800 Myr.

The history of the Precambrian rocks in the Minnesota River valley has some marked resemblances to the events that have been dated³ in the ancient rocks of West Greenland. As in Greenland, the oldest rocks in south-western Minnesota

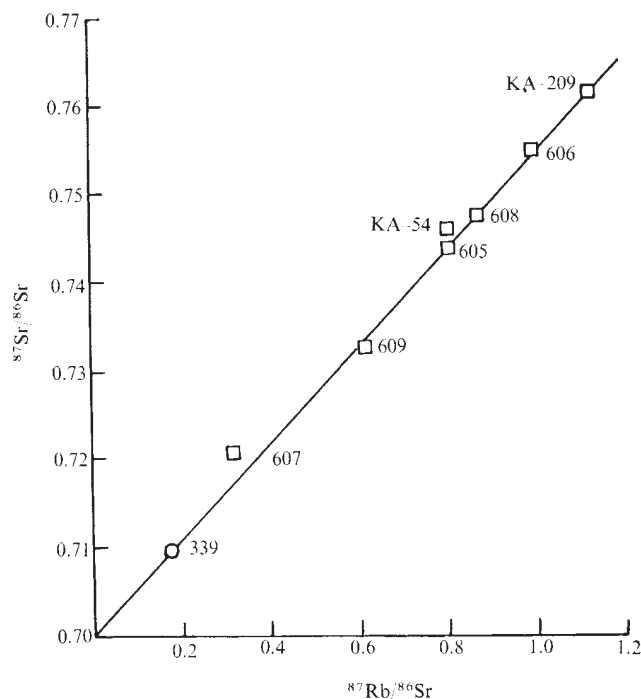


Fig. 2 Whole-rock samples from the foliated phase of the Montevideo Gneiss (□). ○, Morton Gneiss—3,800 Myr (0.700).

are gneisses ranging in composition from tonalite to granodiorite and quartz monzonite. These were intruded by granite and pegmatite of one or more periods of magmatic activity. Preliminary results indicate an age of approximately 3,000 Myr for some of the massive layers of granite which were formerly considered an original component of the Montevideo Gneiss¹. Foliated and massive phases were folded together during the high-grade metamorphic event 2,650 Myr ago, at which time large volumes of granite were emplaced in south-western Minnesota. Basaltic dykes were intruded in several periods, the older ones being involved in the 2,650-Myr metamorphism and predating the granitic activity of approximately 3,000 Myr ago. Some dykes are much younger and were intruded before the emplacement of granite 1,850 Myr ago.

Work is in progress on U-Pb and Rb-Sr dating of the old gneissic rocks and of the younger rock units. The work at Northern Illinois University was supported by a grant from the National Science Foundation. We thank G. R. Himmelberg and R. L. Bauer of the University of Missouri for help in the sampling.

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Anomalies in particle shape during seeded growth of polystyrene latices

We have observed unusual changes in latex particle shape during the 'seeded' growth of polystyrene latices in emulsifier-free systems (Fig. 1a). To our knowledge this has not previously been recorded.

Polystyrene latices may be grown in the absence of emulsifier^{1,2} (Table 1); however, the ultimate size of the latex produced is limited. In an effort to obtain larger particles, using an emulsifier-free technique, we used a two-stage process whereby a latex of selected size was utilised as the 'seed' to grow larger particles.

Table 1 Normal growth process

Time after initiation (min)	Size (nm)	Standard deviation (nm)	B_1	B_2
1	20.3	4.2	1.69	6.02
5	37.5	2.7	0.03	3.04
15	85.3	3.7	0	2.57
30	101.4	2.9	0.05	2.33
60	145	3.3	0.11	2.93
90	176	2.0	0.61	2.45
120	204	4.2	0.14	3.49
175	244	2.0	0.01	2.29
275	308	3.5	0.09	2.92
302	327	4.0	0.09	1.83
330	347	3.7	0	2.35
1,257	435	4.0	0.45	6.59

B_1 and B_2 describe the skewness and kurtosis of the particle size distribution and should be 0 and 3 for normal distribution³.

In a typical seeded growth process, a sample (50 g of 51% w/w polystyrene latex of diameter 520 ± 11 nm) was agitated for 24 h at $70 \pm 0.2^\circ$ C with 25 g of styrene monomer, 54 g distilled water and 0.02079 g potassium persulphate free radical initiator dissolved in 19.5 g distilled water. Samples were taken at intervals and sizes determined by means of electron microscopy (Table 2). The latex particles increased in size, through stages which were themselves highly monodisperse, to a diameter of 606 ± 5 nm, an increase of approximately 20%.

Two hours after the start of the experiment, new polymer particles of diameter about 25 nm appeared. These particles formed agglomerates, which in turn attached themselves to the original polymer seed spheres. Since no small particles were present at the end of the reaction, we inferred that they had contributed to the growth of the 'seed'. After 4 h, incomplete spheres, eroded to varying degrees, were observed and from

Table 2 Seeded growth process

Time after initiation (min)	Size (nm)	Standard deviation (nm)	B_1	B_2
0	520 (520)	11.0 (11.0)	0.05 (0.05)	4.00 (4.00)
22	525	5.0	0.05	4.08
58	526	6.0	0.16	3.75
133	522 (528)	5.0 (4.0)	0 (0.22)	1.99 (2.46)
182	542 (531)	7.0 (5.0)	6.02 (0.54)	2.42 (2.56)
244	542 (540)	4.0 (9.0)	0.62 (1.29)	3.18 (4.09)
301	562	8.0	0.09	2.79
362	589	30.0	3.41	7.72
1,440	625 (606)	6.0 (5.0)	0.33 (0.01)	5.96 (2.07)

Values in parentheses refer to process where all reactants were added and polymerisation begun immediately. See also legend for Table 1.

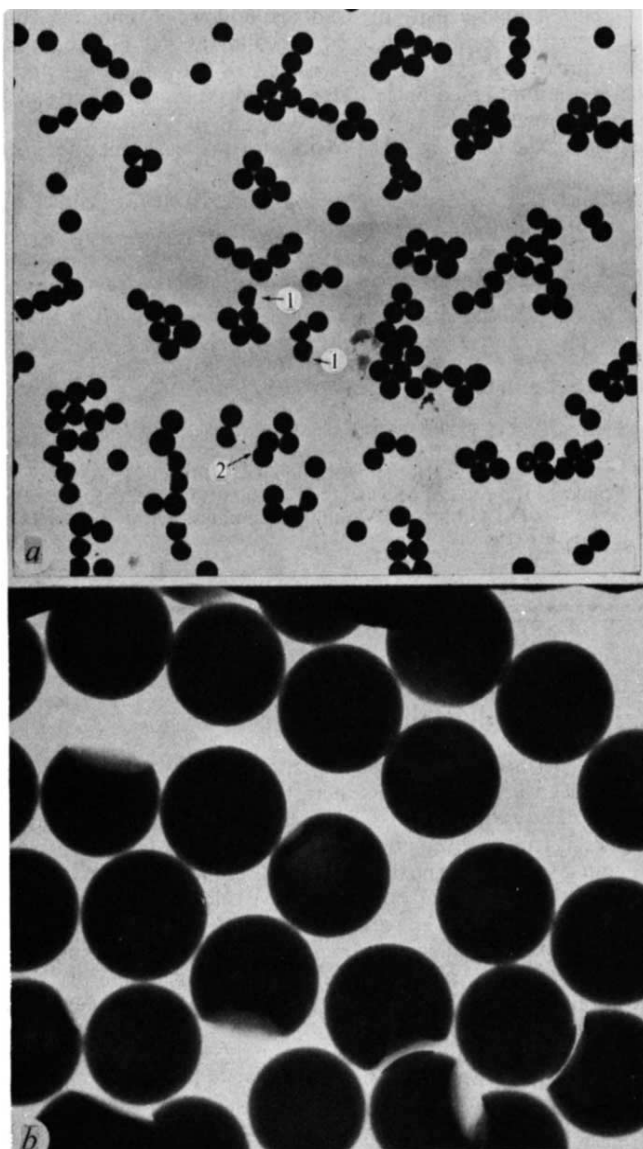


Fig. 1 *a*, Eroded polystyrene latex particles produced during an emulsifier-free seeded growth process. Seed latex 520 ± 11 nm 362 min after initiation of reaction, size 589 ± 30 nm. *b*, Eroded particles at high accelerating voltage (100 KV) to reveal internal detail.

measurements we concluded that, in some cases, erosion must have involved some of the original 'seed'. This dissolution of hardened polymer spheres is difficult to explain. Erosion increased until, after 6 h, the phenomenon was most pronounced with large proportions of some spheres eroded away, for example Fig. 1*a*, arrow 1. At the end of the polymerisation (24 h), however, the erosion was no longer seen, the spheres having apparently reformed.

Detailed examination in the electron microscope at higher accelerating voltage (Fig. 1*b*) revealed the presence of eroded particles with varying orientations and it is possible that the majority of particles was affected. The formation of incomplete latex spheres did not seem to be a consequence of specimen preparation and subsequent electron microscopy since it was not recorded during normal growth processes.

In order to study the possible effect of the monomer on swelling and erosion of the polymer particles, a sample of 'seed' latex was left in contact with monomer overnight at room temperature before the initiator was added and the 24 h polymerisation carried out. A similar abnormal growth process was

observed. (A sample of this particular grown latex (625 ± 6 nm) was stored for 5 months at room temperature after which no further erosion, caused by storage in unreacted monomer, was observed.)

We note that in some cases the incomplete spheres appeared to originate from groups of two or more, where clear interlocking occurred (Fig. 1*a*, arrow 2). Examination of numerous particles, however, failed to reveal any spheres with two or more eroded portions. Such particles might be expected as the result of the break-up of fused spheres.

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Oriented glass ceramic fibre

LONGITUDINAL velocity gradients in a flowing system have a powerful orienting influence on macromolecules and have been used to align polyethylene chains immediately before crystallisation¹ and from the melt². Corning 119 SCR is a glass which crystallises to a glass ceramic containing fluorophlogopite (mica) as the principal crystalline phase. By subjecting it to pure extensional flow during crystallisation, strict alignment of the fluorophlogopite crystals has been achieved. Once produced, the alignment is maintained during subsequent drawing down to fibre diameters of less than 100 μ m.

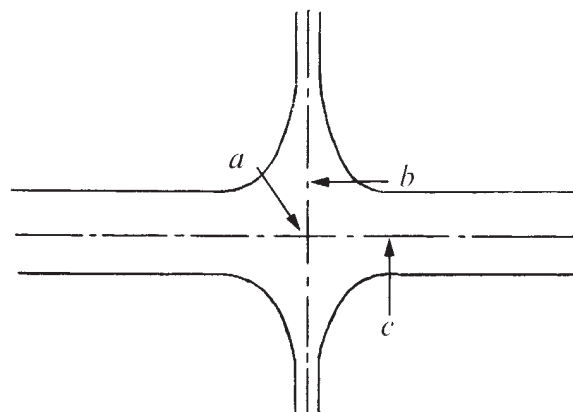


Fig. 1 Flow produced by impinging jets. *a*, Centre of symmetry; *b*, symmetry plane; *c*, symmetry axis.

A large strain rate, free from the complicating effects of rotation, exists along the axis (and in the plane of symmetry) of two opposed fluid jets meeting as shown in Fig. 1 (refs 1 and 3). Along the axis, the fluid velocity is zero at the centre of symmetry, increasing to a velocity v —equal to that for unrestricted flow—at a distance of approximately $d/2$, where d is the bore diameter

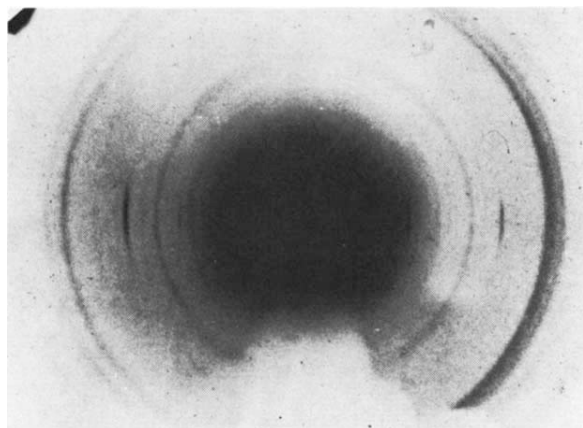


Fig. 2 X-ray pattern from an extruded rod. The rod axis is vertical.

of the jets. The corresponding strain rate is $\dot{\epsilon} \sim 2v/d$; for example, $2 \times 10^3 \text{ s}^{-1}$ for $v = 1 \text{ m s}^{-1}$ and $d = 1 \text{ mm}$.

The same kind of flow field is obtained by extruding fluid through opposed dies; v is then the speed of the fluid leaving the dies and is determined by the speed of the extrusion ram and the bore diameters of the container and dies.

The extrusion press for this experiment consists of a stainless steel container placed inside the coil of a high frequency induction furnace and between the cross head and base plate of a tensile testing machine. Lowering the cross head pushes a pair of rams into two vertical channels which are interconnected by a

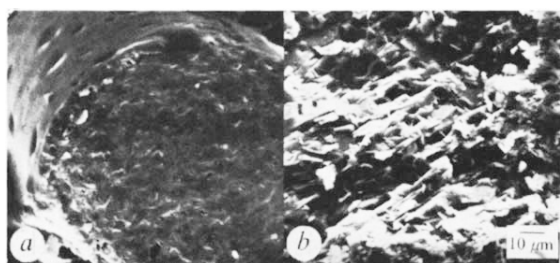


Fig. 3 Scanning electron micrographs of fracture surfaces. *a*, Normal to the axis of a 150 μm diameter fibre drawn from a 2 mm diameter extruded rod; *b*, oblique to the axis of an extruded rod subsequently fully crystallised by annealing.

horizontal channel across the middle of the container. All three channels are filled with glass; the dies are oriented vertically and countersunk into the horizontal channel. Both the radial velocity gradient of the fluid entering the dies and the shearing flow of the fluid within the dies are also expected to have an orienting effect on the crystal nuclei.

Figure 2 shows the Laue transmission X-ray diffraction pattern from a rod extruded at the crystallisation temperature (950°C) using an interdie strain rate of 165 s^{-1} . Fluorophlogopite is hexagonal⁴ and the basal plane reflections 0003 and 0006 each take the form of a pair of arcs parallel to the rod axis. All other reflections occur as Debye-Scherrer rings because the crystals are oriented randomly about the hexad axis. Identical patterns have been obtained from rods extruded and then drawn down to diameters of 150 μm and 75 μm .

The alignment of crystals can be observed directly by scanning electron microscopy of fracture surfaces (Fig. 3*a*). The density of crystals is uniform except very near the fibre surface where it falls to almost zero. In drawn samples, the surface

contains a high density of shrinkage hollows. Completion of the crystallisation treatment, by annealing at 950°C , produces some growth of the oriented crystals plus growth of new and, therefore, unoriented nuclei. This second generation of crystals is evident in Fig. 3*b*.

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The direct measurement of sulphur deposition on bare soil

THREE one-thousandth acre gauges, respectively 20, 40 and 60 inches deep were made at Rothamsted Experimental Station in 1870 by Lawes and Gilbert¹. Since 1870 the soil in the drainage gauges has not received any fertiliser and has not been cropped. Amounts of nitrogen mineralised annually² have fallen to about 7 kg ha⁻¹ and from this we have calculated that sulphur mineralised is about 1 kg ha⁻¹ annually. Chemical analysis showed that the drainage water contains twice as much sulphur as is deposited in rain. A direct measurement of the dry deposition of sulphur was made to see if this satisfactorily accounted for the discrepancy.

Two grams of air-dried sieved soil (0.5 mm), with a total sulphur content of 9.2 $\mu\text{g S g}^{-1}$, were exposed in Petri dishes inside a standard Stevenson meteorological screen at three sites, Rothamsted (Herts.), Woburn (Beds.) and Saxmundham (Suffolk), in mid-February 1974 for 57 to 77 d. Sulphur deposition rates were calculated from the increases in total sulphur content after exposure (Table 1). Air sulphur concentrations were measured at Woburn Experimental Station and Framlingham (near Saxmundham) and identical deposition velocities (V_g) calculated for both. Since, at this time³, the measured deposition rates would be close to the annual means, we have calculated depositions (kg S ha⁻¹) of 18.1 (Saxmundham), 27.5 (Rothamsted) and 65 (Woburn) annually. An independent check on the Rothamsted figure (27.5 kg S ha⁻¹) was made from the analysis of drainage water from the 20-inch deep, one-

Table 1 The period of exposure, increase and deposition rate of sulphur to bare soil at three sites and the air sulphur concentration and deposition velocity for two of the sites

	Saxmundham*	Rothamsted	Woburn
Period of exposure (d)	77	60	57
S increase, g cm ⁻²	38.2×10^{-6}	45.2×10^{-6}	101.6×10^{-6}
S deposition rate, g cm ⁻² s ⁻¹	5.74×10^{-12}	8.72×10^{-12}	20.63×10^{-12}
S air concentration g m ⁻³	13.5×10^{-6}	—†	47.8×10^{-6}
Deposition velocity (V_g)	0.43 cm s ⁻¹	—	0.43 cm s ⁻¹

* S air concentration measured at Framlingham.

† S air concentration not measured at Rothamsted.

thousandth acre drain gauge during 1969, the hundredth year. The SO_4 sulphur collected in drainage through this gauge was $58.36 \text{ kg S ha}^{-1}$. Subtracting sulphur content of rainwater ($33.02 \text{ kg S ha}^{-1}$) we obtain $25.34 \text{ kg S ha}^{-1}$ which represents dry deposition plus a small amount of sulphur mineralised from soil organic matter. Ignoring mineralised sulphur, we have calculated a mean sulphur deposition rate of $8.03 \times 10^{-12} \text{ g cm}^{-2} \text{ s}^{-1} \text{ yr}^{-1}$ which, allowing for differences in air SO_2 concentrations between 1969 and 1974, agrees well with the figure obtained here ($8.72 \times 10^{-12} \text{ g cm}^{-2} \text{ s}^{-1} \text{ S}$).

Deposition velocities of sulphur with respect to vegetation have been shown to be two or three times faster^{4,5} so that, the amount of sulphur deposited on vegetation would be correspondingly greater than the amounts measured here.

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Fluorocarbon anaesthetics break hydrogen bonds

MANY fluorocarbon derivatives that contain higher halogens dissociate hydrogen bonds of the $\text{N-H}\cdots\text{N}$, $\text{O-H}\cdots\text{O}$, $\text{S-H}\cdots\text{S}$, $\text{N-H}\cdots\text{O}=\text{C}$ type which are similar to those occurring in living organisms. A parallelism exists between this property of fluorocarbons and their anaesthetic potency. Their interference with cell division might also be related to it.

Halothane ($\text{CF}_3\text{-CHClBr}$) is the most widely used inhalation anaesthetic¹⁻³. The mechanism of the anaesthetic action of these compounds has not yet been elucidated, although anaesthetic potency has been related to lipid solubility^{4,5}, clathrate-hydrate formation^{6,7}, and interaction with proteins to produce conformational changes⁸⁻¹⁰. We have observed that fluorocarbons can 'break' hydrogen bonds^{11,12} and have investigated the relationships between this property and their anaesthetic properties by measuring infrared spectra of systems which are hydrogen bonded in solvents of chlorine, bromine or iodine containing fluorocarbons. These systems were hydrogen-bonded amides (N -ethylacetamide, N -methylbenzamide, acetanilide, δ -valerolactam), alcohols, amines and thiols. An order of potency of hydrogen-bond breaking has been established from the infrared band intensities: $\text{F} < \text{Cl} < \text{Br} < \text{I}$. This applies to all $\text{N-H}\cdots\text{N}$, $\text{O-H}\cdots\text{O}$, $\text{S-H}\cdots\text{S}$ and $\text{N-H}\cdots\text{O}=\text{C}$ type hydrogen bonds. It is very significant, in our opinion, that $\text{F} < \text{Cl} < \text{Br} < \text{I}$ is also the order of increasing anaesthetic potency^{1,3}.

Another observation deserves consideration. If the fluorocarbon contains a hydrogen, its potency of hydrogen-bond breaking greatly increases. Although $\text{CF}_3\text{-CCl}_3$ has no significant ability to break hydrogen-bonds $\text{CF}_3\text{-CHCl}_2$ is about as potent as halothane (see Fig. 1).

In view of these two parallels between hydrogen-bond breaking and anaesthetic potencies it is natural to speculate that the breaking of hydrogen bonds is an important step in the mechanism of anaesthesia which has been induced by fluorocarbons. The hydrogen bonds that may be 'broken' can be water-water or water-protein bonds or hydrogen bonds within

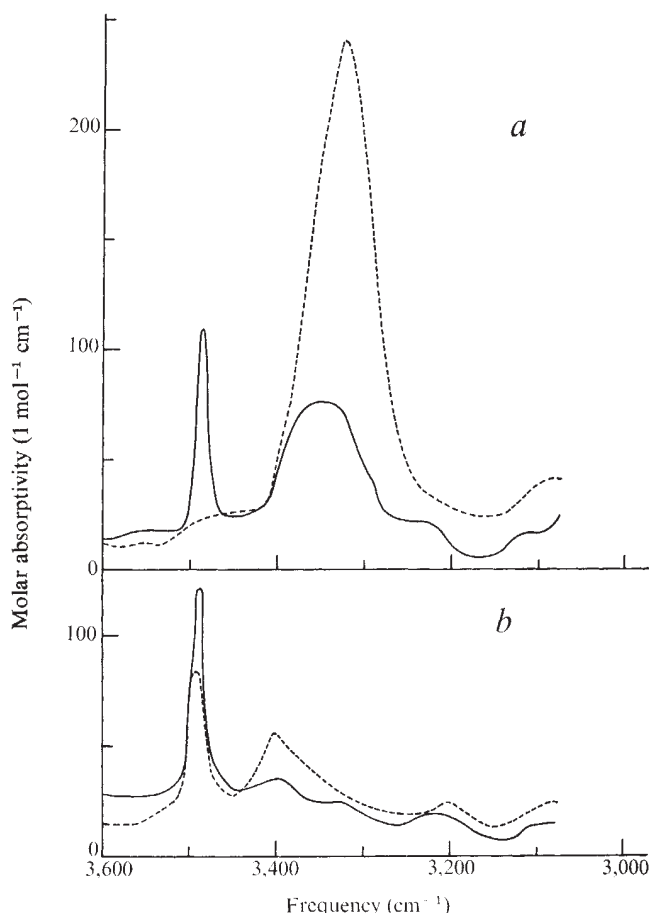


Fig. 1 The infrared spectra of a 0.045 M solution of N -methylpivalamide in a 1:1 mixture of CCl_3F and methylcyclohexane (dashed line) and in the same solvent with 1.02 M $\text{CF}_3\text{-CHCl}_2$ added (solid line). *a*, At -80°C ; *b*, at 23°C .

the protein and nucleic acid structures; they all have energies similar to those of our model systems. In some cases the hydrogen bonds may actually be dissociated, in others a lesser perturbation may be sufficient.

The importance of this observation may well go beyond the mechanism of anaesthesia. Nunn *et al.*¹³, and Jackson¹⁴ have reported that halothane arrests mitosis. Mitosis goes through several phases involving both the breaking and the formation of the hydrogen bonds which keep the two parts of the nucleic acid double helix together. In view of the potent ability of fluorocarbon anaesthetics to break hydrogen bonds it is tempting to speculate that the former interfere with cell division by perturbing or breaking hydrogen bonds around or in the helices, or in the mitotic spindle.

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Radiocarbon chronology for Seibal, Guatemala

THE significance of the study paper reported here is the fact that the origins of Maya civilisation are pushed much further back into the past. Radiocarbon dating of the earliest and latest states of the Mayan 'city' centre of Seibal indicate an origin as early as 900 BC, declining by about 1000 AD. This discovery narrows the interval between the beginning of lowland Maya occupation and the Olmec centres on the Gulf of Mexico, allowing for the coexistence of quite different cultures in relatively close geographical proximity.

Seibal, located on a bluff in the upper Usumacinta-Pasión river drainage of Guatemala (approximately 16°N, 90°W), is one of the important lowland Maya 'city' centres excavated in recent years by Harvard's Peabody Museum^{1,2}. Radiocarbon dating of charcoal and bone samples at the University of California (UCLA) indicate that the city has been in existence for almost 2,000 yr, roughly symmetric about the AD-BC division³. Seibal's time was over by 10.5.0.0.0 katun, equivalent to 928 AD, even though some population lingered on for some time. A more detailed chronology is based upon the following radiocarbon measurements in conjunction, where applicable, with Mayan dates, which in turn are related to our present calendar *via* the Goodman-Martinez-Thompson correlation (Fig. 1). All radiocarbon samples were pretreated to remove impurities and analysed, where appropriate, by their collagen content, precautions being taken for isotopic purity⁴. A comparison showing the concordance of charcoal- and collagen-based radiocarbon dates has appeared elsewhere⁵.

The earliest occupation discovered belongs to the Real Xe phase of the Middle Preclassic Period. In particular, a sample of charcoal was dated associated with pottery vessel 2 in cache 7 of Central Plaza group A. This cache was actually located beneath several preclassic floors and definitely sealed in a Real Xe phase context. It consisted of a cruciform arrangement of pottery and jades. Among the latter was a blood letter resembling an ice pick in shape, a style associated with the Olmec horizon and Olmec sites of the Gulf Coast. These Olmec sites date from as early as about 1300 BC and persist as late as the fifth century BC. For the purposes of this paper the dates have been tree-ring calibrated, based on an earlier publication which did not include this calibration⁶.

Previous archaeological estimates of the date of the Xe ceramic sphere derived from radiocarbon dates at Altar de Sacrificios⁷ and other lines of evidence, were in the range 900-600 BC. The present charcoal date of $2,610 \pm 75$ yr (UCLA: 1437; S-382b) can be calibrated to correspond to an age of 900 BC; this, in a general way, is in line with estimates for the early Middle Preclassic Period of Mesoamerica as a whole^{8,9}. In relation to chronology, Real Xe and the Xe ceramic sphere¹⁰ immediately precede the Mamom ceramic sphere.

After the Real Xe phase, the next date obtained is based on a bone sample from burial 19 found beneath a platform in

front of structure D-3. Associated pottery places this burial in the Tepejilote phase of the Tepeu ceramic sphere. The particular pottery types indicate a Tepeu 2 placement, or, at the earliest, a late Tepeu 1. The radiocarbon date obtained from collagen is $1,490 \pm 320$ yr (UCLA: 1640D; S-1316) corresponding to a calibrated age of 500 AD. Because of the small sample size the statistical error is larger than desirable, which explains why this particular date does not agree with the archaeological estimate of about 675-830 AD. This is based on the 11.16.0.0.0 correlation and ceramic styles¹¹, with a preference for the earlier end of this range.

The next datum is a bone sample obtained from burial 17 located in structure A-2. The structural context indicated an Early Classic dating, although the pottery with the burial pertained to the Tepejilote phase and could very well have been the earliest part of that phase, equivalent to a Tepeu 1 date. The radiocarbon date of $1,420 \pm 95$ yr (UCLA: 1640C; S-1193) corresponded to a calibrated age of 600 AD, in agreement with the inception of Tepeu 1 in the 11.16.0.0.0 correlation.

Burnt human bone from a mass burial (No. 4) in structure A-13 provided the next date. The radiocarbon date is $1,100 \pm 100$ (UCLA: 1,640F; S-410) which can be calibrated to 930 AD. Structure A-13 is an interesting small mound or platform that seems to have been designed to receive a mass interment. It is located immediately next to a large ball-court in the Central Plaza of group A. Perhaps the structure does indeed contain the sacrificed remains of persons who had been in some way involved with the game and actually may have been the defeated team. On the basis of associated potsherds, the mass burial can be dated to the Bayal phase of the site's history. Bayal, which can be placed at about 10.0.0.0.0 to 10.5.0.0.0 (830-928 AD), was a time of considerable ceremonial activity at Seibal including architectural construction and dedication of stelae, with monuments dated as late as 10.3.0.0.0 (889 AD). Characteristic of the Bayal phase is the fine Orange pottery of group Y or Altar series.

Similarly, a bone sample from burial 12, which was found below the patio floor of structure 31A, again confirmed the Bayal phase. The building is a small one on the outer periphery of Seibal, as opposed to the main ceremonial centre. The radiocarbon date is $1,000 \pm 65$, calibrated at 1000 AD (UCLA: 1640A), all the associated sherds belong to the Bayal phase, making the radiocarbon dating late for the Bayal period of 830-928 AD, although it is quite possible that some of the outlying structures at Seibal were occupied for a few decades beyond the estimated date of 10.5.0.0.0 katun (928 AD). Activities in the main ceremonial centre were over by 10.4.0.0.0 or 10.5.0.0.0 but secular life may have continued in the outskirts.

Additional confirmation for the age of the Bayal phase is derived from a bone sample from burial 13, found inside a small structure (26d) also on the periphery. An associated burial and its surrounding sherd also indicate the Bayal phase.

Table 1 Correlation between ceramic spheres, phases and tree-ring calibrated radiocarbon dates at Seibal

Ceramic sphere	Phase	Calibrated radiocarbon date (12)
Boca	Bayal	1050 AD
		1000
		930
Tepeu	Tepejilote	600 (500) AD
Tzakol		—
Chicanel		—
Mamom		—
Xe	Real Xe	900 BC

Early start of Real Xe at Seibal narrows the interval between the beginning of lowland Maya occupation and Olmec sites on the Gulf of Mexico.

The radiocarbon date is 940 ± 70 (UCLA: 1640B) calibrated to 1050 AD, in agreement with previous considerations.

On balance, the calibrated radiocarbon dates agree well with lowland Maya Middle Preclassic and Terminal Late Classic estimates. The latter are consistent with an 11.16.0.0.0 correlation of Maya and Christian calendars. Two dates, one from the earlier late Classic Period, the other from what would appear to be the equivalents of a Tepeu 1 or 2 context, are a little more difficult to reconcile with the 11.16.0.0.0 correlation, suggesting, instead a 12.9.0.0.0 correlation. The latter of these two dates does harmonise with Tepeu 1, as dated in the 11.16.0.0.0 correlation, however, while the statistical error of the former still allows for the same interpretation.

Unfortunately, the Early Classic Period (Tzakol ceramic sphere) is very poorly represented at Seibal and no samples from such a context could be obtained. The Late Preclassic (Chicanel ceramic sphere) is, on the other hand, well represented by structures and pottery at the site although we were unable to find appropriate materials for radiocarbon dating.

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saurus dorsalis) regulates its body temperature close to 38.5°C if placed in a chamber with a temperature gradient³. If this lizard is placed in a temperature chamber in which one end is heated to above the animal's lethal body temperature (50°C) and the other end is maintained at room temperature, the lizard regulates its temperature by moving back and forth between the two sides⁴. Under these conditions, one can determine its high and low set-points (Fig. 1). The central nervous control of temperature in an ectotherm, such as a lizard, and an endotherm, such as the rabbit, appears to be quite similar. For example, both possess a hypothalamus which is thermally sensitive^{5,6}, and lesions in the posterior hypothalamus in both lizards⁴ and mammals⁷ lead to an inability to maintain a high body temperature.

Because of the similarities in the central nervous control of thermoregulation in reptiles and mammals, and because fever in mammals is accompanied by major behavioural adjustments, we suspected that fever could be produced in a reptile. We now report that bacteria that produce fever in a rabbit will produce a similar fever in the lizard *Dipsosaurus dorsalis*.

Lizards weighing 25-60 g (Hermosa Reptile Farm, Hermosa, California) were housed in circular cages and fed meal worms, lettuce and water *ad libitum*. The cages and experimental chambers were kept on a 12-h light and 12-h dark photoperiod. The chamber was heated by a 250 W heat lamp which was also on a 12:12 cycle.

Experiments were carried out in a temperature-controlled room. Each lizard was placed in a wooden box (30 cm $\times$ 140 cm $\times$ 30 cm) of which one end was at the room temperature of 30°C and the other end at 50°C . This high temperature was provided by heating coils taped to the undersurface of the floor of one end of the box. The two sides of the box were separated by a small wire mesh bridge to provide a clear boundary between the two temperature extremes. A copper-constantan thermocouple covered with polyethylene tubing (PE 100) was placed about 3 cm into each lizard's cloaca and taped to its tail; this did not noticeably impair the movement of the lizards. Thermocouples were connected to a Honeywell Elektronik 112 multipoint recorder which recorded the temperature of each lizard ($\pm 0.1^{\circ}\text{C}$) every 30 s.

Aeromonas hydrophila, Gram-negative bacteria pathogenic to reptiles and amphibians⁸, were grown on blood agar and killed by washing in 70% ethyl alcohol. They were then centrifuged and resuspended in physiological saline. The concentration of bacteria was determined by a turbidity test.

Lizards were allowed 1 d to adapt to the wooden box, and on the second day control data were recorded. On the third day either 0.2 ml of a solution containing 2×10^{10} bacteria per ml of saline or 0.2 ml of sterile physiological saline alone was injected into the heart using a 1.5-inch 26-gauge needle. Blood

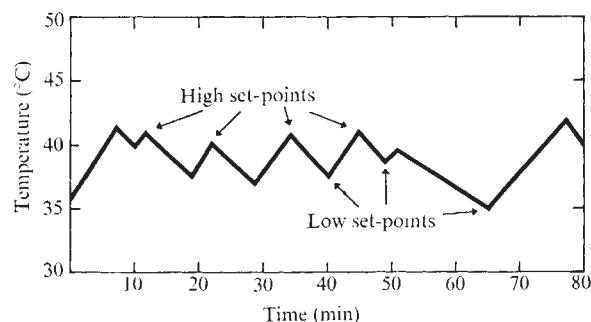


Fig. 1 Record of the cloacal temperature of *Dipsosaurus dorsalis* regulating its temperature in the wooden box in which the substrate at one end was maintained at 30°C and the other end at 50°C . At the high set-point, the lizard moved from the warm side of the chamber to the cool side. At the low set-point the lizard moved back to the warm side of the chamber. Data were recorded every 30 s. The high and low set-points, for any time period, represent the average of these points over that time period.

Fever in the lizard *Dipsosaurus dorsalis*

FEVER is considered to be a universal response of warm-blooded animals to endotoxins¹. Although during a fever a mammal uses behavioural as well as physiological means to increase its body temperature², it is not known whether fever develops in an animal such as a lizard which regulates its body temperature largely by behaviour. For example, the desert iguana (*Dipso-*

was redrawn into the syringe to ensure that the needle was in the heart. All injections were done at the same time of day to avoid possible effects of diurnal variations.

To determine whether increases in body temperature were due entirely to behavioural modifications, or whether an increased internal production of heat was part of the response, three lizards were given injections of bacteria identical to the above. They were then placed in a wooden chamber (14 cm × 30 cm × 30 cm) which was held at 30° C. Cloacal temperatures were measured as before.

Four New Zealand white rabbits (*Oryctolagus cuniculus*) were used to determine whether *A. hydrophila* could also produce fever in mammals. The rabbits' tails were shaved and a thermocouple was inserted 10 cm into the rectum and taped to the tail. The rabbits were placed in a restrainer and allowed 1 h to acclimatise to room temperature (22° C). Then 0.2 ml of saline was injected into the marginal ear vein. After 1 h (control period), 1 ml of 3×10^9 bacteria per ml saline was injected into the marginal vein of the other ear.

In 10 lizards, given free choice of either the 50° C or 30° C environment, injection of 4×10^9 bacteria produced little increase in temperature during the first few hours. Body temperature increased approximately 2° C between the fourth and sixth hours (Fig. 2). The increases of the average high (41.1° C to 42.7° C) and low (37.4° C to 39.7° C) set-points were highly statistically significant ($P < 0.001$ using paired sample analysis).

When the same concentration of bacteria was injected into three lizards maintained in a constant temperature chamber at 30° C, their temperatures did not change throughout the control and experimental periods. Injection of saline into nine lizards, given free choice of the warm or cool environment, produced no changes in high or low set-points (Fig. 2). Comparison of lizards injected with saline to those with *A. hydrophila* revealed a statistically significant increase in the high ($P < 0.02$) and low ($P < 0.003$) set-points during the second 3 h (Student's *t* test). Injection of saline into the four rabbits produced no increase in rectal temperature. Injection of the bacteria produced a fever with a latency of about 20 min and a mean maximum rise of 2.2° C within 3 h.

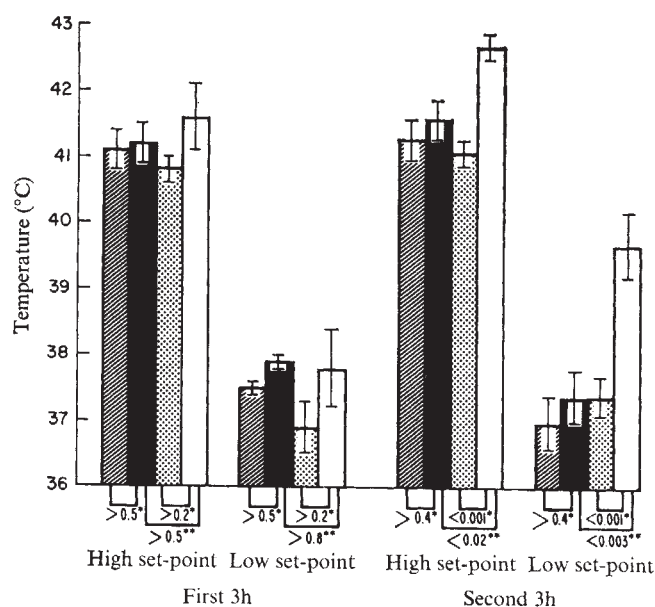


Fig. 2 Average high and low set-points ($\pm$ s.e.m.) during control periods (day 2) and after intracardiac injection of 0.2 ml isotonic saline or 0.2 ml of 2×10^{10} *Aeromonas hydrophila* ml⁻¹ (day 3) into *Dipsosaurus dorsalis*. *, Level of significance using paired sample analysis; †, level of significance using Student's *t* test. Hatched columns, control period before saline injections; solid columns, period after saline injections; stippled columns, control period before *Aeromonas* injections; open columns, period after *Aeromonas* injections.

These data indicate that a bacterium that causes fever in a rabbit has a similar effect in a lizard. Since the lizards could not increase their temperature in response to the bacteria when the ambient temperature was held constant, fever in these lizards cannot be developed by increased internal production of heat. The relatively long latency before the onset of fever in the lizards might be due to the lower metabolic rate of an ectotherm in comparison with that of an endotherm.

These results demonstrate that fever can be sustained by behavioural regulation in an ectotherm. Also, since the hypothalamic control over thermal responses in reptiles and mammals are similar⁴⁻⁶ and as we have shown, a similar concentration of bacteria will produce a similar increase in temperature in both a reptile and a mammal, we suspect a common origin for reptilian and mammalian fever. If this is true, then the ability to develop a fever existed before the time when evolutionary lines for mammals and reptiles diverged. This suggests that at least the behavioural component of fever had evolved by the late Palaeozoic or early Mesozoic, or perhaps even earlier. We cannot rule out the possibility, however, that fever might have independently and perhaps recently evolved in reptiles and mammals.

These findings also open new possibilities for ascertaining the adaptive value of fever. The question of whether fever is beneficial or harmful to the host has been difficult to resolve in mammals. Since the increase in body temperature in response to bacterial infection might have evolved in primitive reptiles, or even in amphibians, the adaptive value of fever might be revealed by a careful study of the role of the febrile response in these classes of vertebrates.

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Dependence of juvenile hormone release from corpus allatum on intraglandular content

THE corpus allatum has been shown to be the source of chemically defined juvenile hormones in five different species of insect¹⁻⁵. The release of juvenile hormone from active corpora allata is known to be a prerequisite for the rapid induction and promotion of vitellogenesis in adult female insects of several orders, including all tested members of the Orthoptera^{6,7}. It is generally believed that temporal patterns of activity in the adult female corpus allatum are of prime importance in initiating and maintaining oocyte growth^{6,8}.

Up to now the activity of the corpus allatum has been inferred from indirect observations such as glandular activity

after transplantation into a suitable host animal⁹, gland extirpation plus hormone replacement therapy¹⁰ and measurement by bioassay of apparent titres of juvenile hormone in the haemolymph^{11,12} and in freshly excised glands¹². A direct, quantitative method for estimating the activity of the corpus allatum has recently become available, following the demonstration that the synthesis and release of juvenile hormone from corpora allata taken from adult female *Schistocerca gregaria* can be rapidly measured *in vitro* by precise radiochemical procedures^{4,13}. Here we deal mainly with the possible regulation of release of newly synthesised juvenile hormone using corpora allata from female *S. gregaria* during the first gonotrophic cycle. We show that at all times during sexual maturation it is the rate of synthesis of juvenile hormone and not the rate of release, which determines the endocrine activity of the glands.

To explore the relationship between the rate of synthesis of locust juvenile hormone, methyl 10,11-epoxy-3,7,11-tri-methyl-2,6-*trans,trans*-dodecadienoate ($C_{16}JH$), the extent of its storage within the glands and its rate of release, freshly-isolated corpora allata were incubated in TC 199 medium containing methyl-¹⁴C-methionine for 2 h at 30°C with shaking. The medium was separated from the washed glands and both were analysed for radiolabelled $C_{16}JH$ content as previously described¹³. Observations were made on glands taken daily from adult female locusts between the ages of 3 and 12 d after fledging, during which period there are changes of approximately 400-fold in the rate of juvenile hormone release measured *in vitro* (S. S. T., and G. E. P., unpublished). We also carried out parallel measurements on glands incubated with optimal concentrations of C-2³H-*trans, trans* farnesenic acid, an exogenous precursor known to stimulate directly the rate of juvenile hormone biosynthesis in these glands⁴. Thus, on each day of sexual maturation, we compared the steady state glandular content of $C_{16}JH$ with its rate of release from the glands, both in glands synthesising juvenile hormone spontaneously and in those whose rate of synthesis has been stimulated artificially with exogenous farnesenic acid. In this way we tested by direct observation, the alternative hypotheses that the naturally variable rates of release of hormone from the glands are the result either of direct regulation of the rate of biosynthesis, or of the direct regulation of the rate of release of newly synthesised hormone. Figure 1 shows the rates of release of hormone as a function of intraglandular content. For each day the points representing spontaneous activity and farnesenic acid-stimulated activity have been joined with a straight line, whose slope we term the 'release coefficient'. These slopes indicate the extent to which stimulating JH biosynthesis, by addition of farnesenic acid to the medium, alters the relationship between glandular content and rate of release. It can be seen that the data approximate a linear relationship of 'rate of release' on 'glandular content'; analysis shows that the regression line passes through the origin and has a slope of 2.7 h⁻¹. Moreover, this mean regression is reflected in the 'release coefficients' of the various glands having these widely different spontaneous activities (mean = 2.83 h⁻¹; s.d. ± 1.00). Although the variations in slope are small compared with the variations in spontaneous activity of the glands, the possibility existed that the observed variations in 'release coefficient' might have some functional relationship with the spontaneous *in vitro* activity of the glands. We have therefore compared these two parameters (Table 1) and regression analysis indicated a very weak correlation (slope = 0.046; s.d. ± 0.023) indicating that differences in 'release coefficient' are attributable to independent variations including experimental error.

Thus at all times during sexual maturation, both the rates of synthesis and release of juvenile hormone can be stimulated proportionately by the addition of farnesenic acid to the medium, such that the hourly release of newly-synthesised juvenile hormone is approximately equal to 2.7 times the steady state glandular content of the hormone. This seemingly simple relationship between the rate of release of juvenile

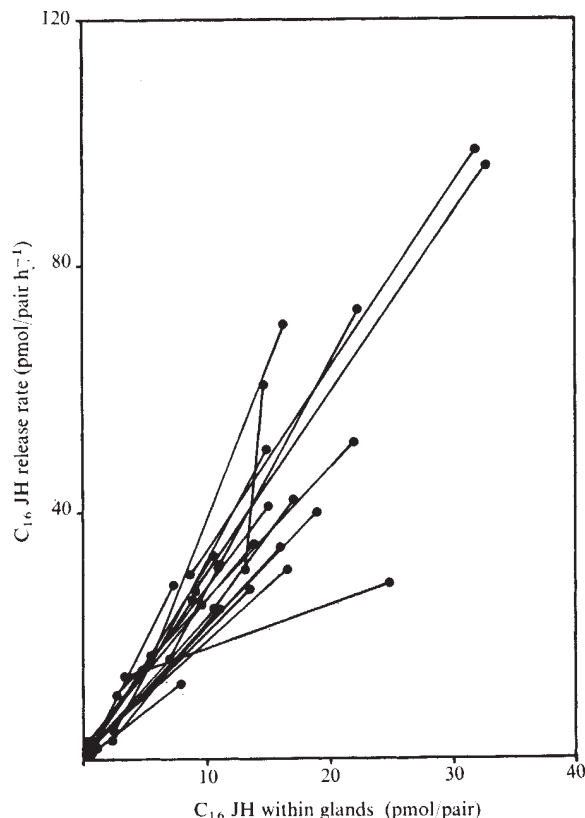


Fig. 1 The rate of release of $C_{16}JH$ relative to its concentration within the corpora allata. Each point represents the mean of two sets of observations on single pairs of glands from adult female locusts during the first gonotrophic cycle. The lines join observations from glands synthesising $C_{16}JH$ spontaneously and those stimulated with ³H-farnesenic acid on each given experimental day. In every case the rate of release from stimulated glands is higher than the spontaneous rate. Both the rate of release and the glandular content of hormone were monitored throughout the period of sexual maturation by incubation of glands for 2 h in TC 199 (20 mM HEPES) containing Ficoll (20 mg ml⁻¹) and either ¹⁴C-methionine (final specific radioactivity 36.7 mCi mmol⁻¹) or ¹⁴C-methionine plus ³H-farnesenic acid (20 μM; specific radioactivity 25 mCi mmol⁻¹). After incubation, individual pairs of glands were separated from the medium and both glands and medium were separately extracted with fifteen volumes of CHCl₃:C₂H₅OH (5:1) after adjustment to pH 4.6 and addition of appropriate nonradioactive marker compounds. Extracts were analysed by thin layer chromatography (Merck F₂₅₄ silica gel) in ethyl acetate: benzene (25:75) followed by episcopic fluorescence quenching densitometry and scanning gas-flow radiometry. Radioactivity in $C_{16}JH$ was quantified by liquid scintillation spectrometry. At all times the rate of release of juvenile hormone is determined by the glandular content of hormone. ($\alpha = 0.0$ pmol h⁻¹ per pair of glands; $\beta = 2.7$ h⁻¹; $r = 0.92$).

hormone and the steady state glandular content, irrespective of the spontaneous activity of the glands, suggests that the export of juvenile hormone is governed by laws of simple physical diffusion. Although the route of export of juvenile hormone is not yet established, it has been proposed that this may involve diffusion out of the glands through a network of extracellular spaces⁹.

Ultrastructural observations on the corpora allata of this¹⁴ and other^{9,15} insect species have led others to suggest an association between the release of juvenile hormone and observable changes in the subcellular architecture of the glands. It has been suggested that in the corpus allatum of *Calliphora erythrocephala* newly synthesised juvenile hormone accumulates initially in vacuoles associated with the agranular reticulum. These vacuoles eventually break down into lipid droplets

Table 1 Relation between rate of release of $C_{16}JH$ synthesised spontaneously and its 'release coefficient'

Rate of release of $C_{16}JH$ synthesised spontaneously (pmol h ⁻¹ per pair of glands)	Release coefficient $\frac{R_{st}-R_{sp}}{C_{st}-C_{sp}}^*$	Adult age (d)
29.90	3.28	9
16.70	5.11	12
14.63	2.92	8
12.09	3.80	10
10.40	2.11	8
4.70	2.16	9
4.40	3.68	10
3.08	2.34	7
2.80	2.64	7
2.03	1.95	6
1.83	1.75	12
1.80	2.07	6
1.80	3.88	4
0.80	2.85	5
0.44	2.86	6
0.43	2.30	5
0.14	2.01	5
0.11	2.93	4
0.07	3.15	4
0.05	2.88	3

Each value represents the mean of two separate sets of observations on single pairs of glands from animals of the age indicated. There is no correlation between spontaneous rate of hormone release and 'release coefficient' ($\alpha = 2.59$, s.d. ± 0.21 ; $\beta = 0.046$, s.d. ± 0.023 ; $r = 0.43$).

* $R_{st} = C_{16}JH$ release rate of glands stimulated by addition of 3H -farnesenic acid; $R_{sp} = C_{16}JH$ release rate of glands synthesising hormone spontaneously; $C_{st} = C_{16}JH$ content of glands stimulated by addition of 3H -farnesenic acid; $C_{sp} = C_{16}JH$ content of glands synthesising hormone spontaneously.

within the cytoplasm and are then released into the haemolymph¹⁵. On the other hand, in *Locusta migratoria*, the fragmentation of the smooth endoplasmic reticulum is believed to be an important stage in the release of hormone from the glands⁹. It has also been reported that corpora allata of *S. gregaria* contain lipid droplets which increase in size towards the periphery of the cells¹⁴. It is difficult to reconcile the results presented here with any proposed mechanism of hormone release involving massive changes in the ultrastructural organisation of the cells, particularly since it is known that in *S. gregaria* newly synthesised juvenile hormone can be detected in the culture medium within 10 min of the addition of radiolabelled precursors¹³. Therefore we propose that the observed changes in the ultrastructure of cells of the corpus allatum reflect changes in their hormone synthetic capacity, rather than reveal the morphological basis of hormone synthesis, intracellular transport and release.

The possibility that storage of newly synthesised juvenile hormone may be an important facet of the normal endocrine function of the corpus allatum has never been directly tested in any species of insect. It is now clear that at no time during the course of rapid sexual maturation in the adult female desert locust do isolated corpora allata synthesise juvenile hormone and then fail to release it into the medium. Whether or not this also holds true in circumstances other than those of rapid sexual maturation remains to be seen.

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Choline transport by neuroblastoma cells in tissue culture

NEUROBLASTOMA cells in tissue culture have been used widely as an *in vitro* model for neural functions. Certain 'cholinergic' clones have raised choline acetyltransferase activities¹, and all clones studied display a high affinity uptake system for exogenous choline². Whereas low affinity ($K_T \approx 10^{-3}$ M) choline transport systems occur in various neural and non-neural mammalian tissues, high affinity ($K_T < 10^{-5}$ M), sodium-dependent choline uptake in brain synaptosomes^{3,4} and the guinea pig intestine (C. B. Pert, and S. H. Snyder, personal communication) is restricted to cholinergic neurones. Efficient conversion of choline to acetylcholine, ionic requirements and sensitivity to inhibitors should uniquely distinguish the cholinergic transport system from those of similar affinity for choline which occur in many cultured mammalian cell lines. We have examined these properties of the choline transport system in cholinergic and non-cholinergic neuroblastoma clones.

The profiles for enzymes involved in the synthesis and degradation of acetylcholine are characteristic of each cloned neuroblastoma cell line (Table 1). The activities of choline acetyltransferase which we obtained are similar to other values previously reported for these lines¹, although the N18 clone is

Table 1 Acetylcholinesterase and choline acetyltransferase activities of neuroblastoma cell lines

Cell line	Acetylcholinesterase nmol per mg protein per min	Choline acetyltransferase nmol per mg protein per h
NS20 clone	218	6.50
N18 clone	190	3.71
N1E clone	41	n.d.

n.d., Not detectable.

Neuroblastoma cells were obtained from Microbiological Associates (clone N18) or from Dr Arthur Blume (clones N1E and NS20). The nomenclature used for the clones is that of Amano *et al.*¹. The lines were maintained by serial cultivation in Dulbecco's modification of Eagle's medium supplemented with 10% foetal calf serum. For enzyme assays and metabolic studies, the cultures were allowed to reach stationary phase, giving about 2×10^7 cells per 75 cm² plastic flask (Falcon Plastics, Inc.). Acetylcholinesterase was measured by a modification of the method of Ellman *et al.*¹¹ in which we use 1% Nonidet P-40 to solubilise the enzyme and assay activity at 35°C. Choline acetyltransferase activity was determined by a standard technique¹².

Table 2 Kinetic constants for choline transport by neuroblastoma cells

Constant	NS20		Cell line N18		N1E	
	Unstarved	Starved	Unstarved	Starved	Unstarved	Starved
K_T ($M \times 10^6$)	20.7	4.5	17.1	3.5	10.2	2.1
V_{max} ($M \times 10^{12}$ per min per mg protein)	19.8	21.2	38.9	11.2	2.8	3.0

Neuroblastoma cells of the clones indicated were grown to saturation in Linbro microwells (about 0.4 mg of protein or 8×10^5 cells per well). Triplicate cultures were either incubated overnight in Hank's BSS to deplete the cells of choline and labile choline metabolites or they were merely washed twice with the buffer. For measurement of choline transport, cultures were exposed to 0.2 ml of Hank's BSS containing the appropriate concentrations of 3H -choline (from Amersham Searle at a specific activity of 0.5 Ci mmol^{-1}) for 4 min at 37°C and then quickly washed three times with ice-cold buffer. Cells were lysed by addition of 0.5 ml of distilled water, and radioactivity in the suspension was determined by scintillation counting in dioxane based phosphor. Choline accumulation was linear for nearly an hour in all three clones using both choline depleted cells and those maintained in medium containing choline. After a 1-h exposure to $5 \times 10^{-8} \text{ M } ^3H$ -choline, the concentration of labelled compounds within cells exceeded the radioactivity in the medium by a factor of 20. The kinetics of accumulation and cell/medium ratios were very similar to those reported for lymphoblasts *in vitro*⁷. It was estimated that at the highest choline concentration used, passive diffusion of choline into cells accounted for approximately 5–10% of the total uptake. After correction for diffusion, the data were plotted and linear regression lines calculated. The difference between K_m values obtained with and without choline depletion is highly significant ($P < 0.001$) while the difference between the V_{max} values is not ($0.3 > P < 0.5$).

apparently not 'inactive' in respect of synthesis of this enzyme. We consistently found higher cholinesterase activities in induced cultures of our clone N18 and lower activities in our clone N1E than are usually reported^{1,5}, especially when cells of the N18 line were induced for acetylcholinesterase by leaving serum out of the growth medium⁶. These differences may be attributed to variations in the assay procedure or growth conditions and would not be expected to influence the results of the present study.

K_T values for choline transport were determined in three clonal lines with and without previous choline depletion (Table 2). The affinities of the transport systems for choline in all three clones are quite similar and are increased about fivefold by choline depletion. The K_T values for starved and unstarved cells are similar to those reported for hepatoma cells by Plagemann^{8,9} and for starved neuroblastoma cells². Experiments using choline concentrations as high as $2 \times 10^{-3} \text{ M}$ did not reveal a K_T of lower affinity in depleted cells so it must be assumed that there is only one transport system for choline in neuroblastoma cells and that its affinity depends upon previous exposure of the cells to choline.

V_{max} values for the adrenergic clone (N1E) indicate a lower capacity of its transport system, a reflection, perhaps, of smaller surface area as a result of relatively poor axonation by this clone. In general, the data are consistent with those obtained using hepatoma cells.⁹ The V_{max} values with and without choline depletion are similar to those previously observed in neuroblastoma cells². Double reciprocal analysis of rates of uptake by each of the 3 clones indicated competitive kinetics. This apparent competitive inhibition cannot be due to isotope dilution by leakage of non-radioactive free choline from unstarved cells since there is not sufficient intracellular free choline to produce the observed effect.

We then studied the fate of choline transported into the various clones (Table 3). Only very small amounts of choline were detected in any of the clones. This is in marked contrast to brain cholinergic synapses which accumulate choline and acetylcholine in roughly equal proportions^{3,4} when exposed to a similar concentration of free choline. The formation of small quantities of acetylcholine was, however, significantly dependent on the activity of intracellular choline acetyltransferase (Table 1), being low in the adrenergic clone (N1E), intermediate in the 'inactive' clone (N18) and highest in the cholinergic clone (NS20). The major radioactive product in all 3 clones was phosphorylcholine which, as suggested by data obtained with the long incubation period, was capable of serving as a precursor of phosphatidyl choline. Subsequently, more detailed experiments showed that the free choline could be chased first into phosphorylcholine and then into phosphatidyl choline.

Disposition of choline in this manner would be expected of

cells lacking either the 'high affinity' choline transport system itself or proper coupling to choline acetyltransferase. To distinguish between these possibilities, we examined the effects of various known inhibitors of transport on uptake and metabolism of choline. Synaptosomal uptake of choline at low concentrations is sensitive to hemicholinium³ and dependent on sodium⁴. As Table 4 shows, the neuroblastoma transport system is not inhibited by even a high concentration of hemicholinium and is independent of sodium. The distribution of choline among its various metabolites is also unaffected by these treatments. It seems that although cultured neuroblastoma cells have a transport system for choline, it is similar to that found in many types of non-neural cells and does not display the characteristics associated with the 'high affinity' uptake system unique to brain cholinergic synapses.

Table 3 Fate of choline in neuroblastoma cell lines

	4-min Incorporation			12-h Incorporation		
	N18	NS20	N1E	N18	NS20	N1E
Choline	3.6	2.8	4.1	0.5	1.0	1.3
Acetylcholine	0.2	0.5	0.1	0.1	0.4	0.1
Phosphorylcholine	96.2	96.7	95.9	72.2	71.0	85.2
Phosphatidyl choline	0.1	0.1	0.1	27.2	27.7	13.5
Total	100.1	100.1	100.2	100.0	100.1	100.1

Neuroblastoma cells were grown to confluence in 75 cm² culture flasks (Falcon Plastics, Inc.) and depleted of choline by incubation overnight in Hank's BSS containing an irreversible inhibitor of AChE⁸. 3H -choline was added at a final concentration of $5 \times 10^{-8} \text{ M}$ and incubation continued for the indicated times. Monolayers were washed three times with ice-cold buffer and then scraped off into 0.5 ml of ice-cold 95% ethanol containing 0.2% acetic acid, 2 mM acetylcholine, 3 mM choline and 0.1 mM neostigmine⁸. The suspension was homogenised thoroughly and the debris pelleted at 1,000g for 10 min. The pellet was re-extracted with acid ethanol and then with ethanol-ether (1:1). The ethanol-ether extract was shown chromatographically to contain mostly phosphatidyl choline and a very small amount of phosphoryl choline¹³. Phosphoryl choline, acetylcholine and free choline were all soluble in the acid-ethanol extract but could be separated by high voltage paper electrophoresis¹⁴. Since phosphoryl choline remained at the origin in our electrophoretic system, its identity was confirmed by thin layer chromatography⁸. In 4 min, an average of 4,000 c.p.m. per mg protein were incorporated, increasing to approximately 10,000 c.p.m. per mg protein after 12 h. Uptake by the various lines was quantitatively similar so, to facilitate comparisons, data are expressed as % total c.p.m. recovered. Assuming that complete equilibration of the acetylcholine and phosphorylcholine pools with exogenous labelled choline occurred during 12-h incubation, then the intracellular acetylcholine concentration in NS20 can be calculated to be about 10^{-7} M and that of phosphorylcholine about $2 \times 10^{-6} \text{ M}$. Under the conditions of these incubations, greater than 99% of acetylcholinesterase activity was inhibited. In the absence of an acetylcholinesterase inhibitor even less acetylcholine was recovered, but an accurate determination of the acetylcholine concentration under these conditions could not be made.

Table 4 Effect of various inhibitors on choline transport

Inhibitor	Choline uptake (% of control)
None	100
Hemicholinium (0.1 mM)	131
Na ⁺ replaced by Li ⁺	81
Na ⁺ replaced by sucrose (0.25 M)	66
Ca ²⁺ replaced by Mg ²⁺	93
2,4-dinitrophenol (1 mM)	40
Ouabain (1 mM)	38
Incubation at 0° C	10

Neuroblastoma cells (NS20 clone) were grown to confluence in 75 cm² plastic flasks and depleted of choline metabolites as described for Table 3. The cultures were preincubated with the anticholinesterase agent and the indicated metabolic inhibitor in Hank's BSS for 15 min. Then 2×10^{-3} M ³H-choline was added and after a further 15 min incubation the cells were collected as described in Table 2.

As would be expected for any energy-dependent cellular process, uptake is inhibited by low temperature and 2,4-dinitrophenol. But this should not be interpreted as evidence that choline is taken into neuroblastoma cells by active transport. We have never observed an intracellular choline concentration greater than that in the medium and inhibition of transport by such nonspecific agents could have other explanations. In erythrocytes there is a facilitated diffusion system for choline¹⁰ and we have no evidence against the operation of such a mechanism in neuroblastoma. These inhibitor effects do not eliminate the possibility that the characteristic properties of cholinergic choline transport emerge when a universal uptake system is coupled to choline acetyltransferase. If coupling were absent in neuroblastoma cells, choline kinase and acetyltransferase could compete for their common substrate.

Although the absence of the brain synaptosomal high affinity choline transport system may simply be correlated with the absence of synapses in neuroblastoma cultures¹⁵, there are other explanations of our data. In particular, synthesis of choline acetyltransferase may not be an expression of more generalised cholinergic potential of the NS20 clone. This view is supported by the existence of acetylcholine receptors in neuroblastoma cells¹⁶, a finding that suggests a postganglionic origin for the cells and seems incompatible with the concomitant presence of cholinergic synaptic structures. Nevertheless, the generation of a high affinity uptake system possessing such properties as preferential synthesis of acetylcholine and sensitivity to appropriate inhibitors could provide a convenient biochemical tool for investigating the differentiation of neuroblastoma cells *in vitro*.

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Binding of interferon to gangliosides

RECENT evidence suggests that the antiviral action of interferon is triggered by interaction with the cellular membrane. Mouse interferon covalently bound to Sepharose beads (IF-Sepharose) retains its antiviral potency and only direct contact with these particles produces the antiviral effect^{1,2}. Preincubation of mouse L cells with *Phaseolus vulgaris* phytohaemagglutinin (PHA) blocks interferon action³. The inhibitory action of PHA can be almost completely reversed by washing PHA-treated cells with fetuin, a glycoprotein of high affinity for this plant lectin³. These data suggest that membrane sites interacting with interferon are carbohydrate-containing molecules that bind to PHA, although other explanations for the inhibitory action of PHA based on nonspecific steric or charge effects might be possible. To substantiate further that glycoside-containing membrane components bind to interferon, we have investigated the effect of gangliosides on interferon binding and action.

As Table 1 shows, preincubation of IF-Sepharose with a ganglioside mixture from bovine brain completely blocked antiviral activity. When tested individually, mono- as well as di- and trisialogangliosides were inhibitory. The concentration in the preincubation mixture resulting in maximal inhibition of antiviral activity was lowest for G_{M2} and G_{T1}, suggesting somewhat stronger binding of these two gangliosides. Thin-layer chromatography on silica plates in chloroform-methanol-water (65:45:9) followed by resorcinol spray⁴ revealed one band for G_{M2}, but showed that commercial G_{M1}, G_{D1a} and G_{T1} were somewhat contaminated with other gangliosides although free of G_{M2}. In separate experiments using G_{M1}, G_{D1a} and G_{T1} which were 82–87% pure, essentially similar results were obtained, though G_{M1} and G_{D1a} appeared slightly less inhibitory. G_{M3}, a mixture of the acetyl and the glycolyl neuraminic acid derivatives, was only tested at one concentration because of the small amount available. Its inhibitory action appeared slightly less than that of G_{M2} and G_{T1}, but more than that of the other two gangliosides. In our determinations of viral yield only differences greater than twofold were considered significant. Therefore, although Table 1 compares µg quantities of gangliosides instead of µmol, the accuracy of the assay does not allow conclusions based on molecular weight differences of individual gangliosides. Inhibition of IF-Sepharose by individual gangliosides was the same regardless of whether preincubation was carried out for 1 h or 18 h. When the temperature of the preincubation mixture was varied, however, there was a consistent increase of the inhibitory effect of gangliosides with increasing temperatures. Thus, EMC yield by plaque-forming units (PFU) in response to IF-Sepharose preincubated with mixed gangliosides at 4° C was one-third of that found after preincubation at 37° C. Washing ganglioside-treated IF-Sepharose with PHA solutions partially reversed ganglioside inhibition, suggesting that PHA and interferon have common binding sites (Table 2). It was not possible completely to restore antiviral activity, however, indicating that under the conditions

Table 1 Inhibition of interferon-Sepharose by gangliosides

Ganglioside in preincubation mixture	Viral yield after preincubation of IF-Sepharose with different amounts of ganglioside (μg)					
	400	200	80	40	20	0
Ganglioside mixture	4,096 (4,096)	ND	512	ND	64	64 (4,096)
G _{T1}	ND	ND	2,048	256	64	32/64 (2,048)
G _{D1a}	ND	1,024	256	128	64	32/64 (2,048)
G _{M1}	ND	2,048	256	64	32	32/64 (2,048)
G _{M2}	ND	2,048	2,048	256	64	32/64 (2,048)
G _{M3}	ND	ND	256	ND	ND	4/8 (512)

Interferon covalently bound to Sepharose (IF-Sepharose), prepared as described previously^{1,2}, was treated with gangliosides by incubating 5×10^4 beads with 0.4 ml ganglioside solution in PBS for 75 min at 25°C. The beads were then washed once with 4 ml PBS and suspended in 1 ml of Eagle's minimal essential medium plus Hanks salts without serum (MEM). The ganglioside mixture was obtained from bovine brain (Sigma, type III). Individual gangliosides were obtained from Supelco Inc. Mouse L cells were cultivated in 35 mm plastic dishes (5×10^5 cells per dish) in MEM, containing 10% calf serum. After removal of medium the cells were incubated for 5 h at 37°C with a 1-ml suspension of IF-Sepharose or control Sepharose 4-B in MEM at a ratio of one bead per 10 cells. After removal of the beads, the cells were challenged with encephalomyocarditis virus (EMC) at a multiplicity of infection of 0.1. Viral yield was determined after 16 h of incubation at 37°C by haemagglutination of human red blood cells of type O in serial twofold dilutions of virus suspensions¹⁰. The numbers represent the reciprocal of the highest dilution that showed haemagglutination. The individual gangliosides were designated according to Svennerholm¹¹. Numbers in brackets represent experiments in which control Sepharose 4-B was used instead of IF-Sepharose. ND, Not done.

Table 2 Effect of PHA on ganglioside inhibition of interferon-Sepharose

Ganglioside in preincubation mixture	Viral yield without ganglioside pretreatment of IF-Sepharose	Viral yield after ganglioside pretreatment of IF-Sepharose	
		Before PHA	After PHA
Ganglioside mixture (400 μg)	64	4,096	512/1,024
G _{M2} (80 μg)	32/64	2,048	512
G _{M1} (200 μg)	32/64	2,048	512
G _{D1a} (200 μg)	32/64	1,024	512
G _{T1} (80 μg)	32/64	2,048	512

After treatment of IF-Sepharose beads with gangliosides as described under Table 1, they were suspended in 0.3 ml of PHA (phyto-haemagglutinin from *Phaseolus vulgaris*, M form, Grand Island Biological Co.) at a protein concentration of 4.1 mg ml⁻¹. After incubation for 60 min at 25°C the lectin solution was removed; the beads were washed once with 4 ml PBS, and suspended in 1 ml MEM without serum. Further experimental details were as described under Table 1.

used the affinity of gangliosides for interferon is greater than for PHA. Preincubation of L cells with gangliosides did not result in stimulation of the antiviral effect of interferon, as might be expected if gangliosides became incorporated into the cell membrane to produce additional receptor sites. Such stimulation of activity was observed with cholera toxin⁵, which has been shown to bind strongly to G_{M1} (ref. 6).

Incubation of interferon solutions with gangliosides bound covalently to a Sepharose-polylysine copolymer as described by Cuatrecasas⁷ resulted in complete adsorption of antiviral activity to these beads (Table 3). In contrast, under identical conditions control beads that only contained covalently bound polylysine did not bind interferon to any great extent. Binding of interferon to Sepharose-bound gangliosides was prevented by preincubation of these beads with PHA, in accord with previous results suggesting that PHA and interferon bind to common constituents on the cell membrane³. Washing PHA-treated ganglioside beads with fetuin, a protein of known affinity for PHA which reversed PHA inhibition of interferon action³, failed to restore measurable interferon binding to ganglioside beads, even at a concentration of 60 mg ml⁻¹. It appears, therefore, that PHA has a higher affinity for Sepharose-bound gangliosides than for fetuin in solution. Ganglioside-Sepharose beads also completely inhibited the action of soluble interferon, as Table 4 shows.

Gangliosides are common constituents of cellular membranes. The occurrence of disialoganglioside (G_{D1}) and haematodiside (G_{M3}) in the cell membrane of L cells has been reported⁸. Inhibition of mouse interferon action by preincubation of

Table 3 Binding of interferon to ganglioside-Sepharose beads

Interferon preincubated with	Interferon titre in supernatant
Nothing	1,024
Control beads	1,024
Control beads	
Pretreated with PHA	1,024
Ganglioside beads	< 16
Ganglioside beads	
Pretreated with PHA	1,024

Mouse interferon was prepared by induction of mouse L cells with ultraviolet-irradiated Newcastle disease virus in serum-free MEM plus Earle's salts as described before¹². Crude interferon solutions were concentrated tenfold by pressure dialysis at pH 3 and used as such after changing the pH to 7. They contained 0.2 mg ml⁻¹ protein¹³. Polylysine (molecular weight 30,000, Sigma) was coupled to CNBr-activated Sepharose (Pharmacia) as described by Sica *et al.*¹⁴. Mixed gangliosides from bovine brain (type III, Sigma) were attached to polylysine-Sepharose as described by Cuatrecasas⁷. Covalently bound gangliosides correspond to 0.15 mg ml⁻¹ packed beads, as judged from the amount of carbohydrate in the soluble portion of the reaction mixtures after completion of the reaction¹⁵. In the experiments with PHA, 0.2 ml of packed beads was incubated for 30 min with 0.5 ml lectin solution (4.1 mg ml⁻¹ protein, see Table 2) for 30 min at 25°C. They were then washed twice with 1 ml of 1 M NaCl and twice with 1 ml of PBS. In each of the experiments listed 0.2 ml packed beads (washed twice with 1 ml of NaCl and twice with 1 ml of PBS immediately before use) were suspended in 0.5 ml of interferon solution and incubated for 45 min at 25°C with occasional stirring. The supernatant of the beads was collected, and the beads were washed twice with 1 ml of 1 M NaCl and twice with 1 ml of PBS. Original supernatant and washings were combined and the interferon titre was measured by determining the cytopathic effect in serial twofold dilutions on mouse L cells after infection with vesicular stomatitis virus (VSV). Control beads represent polylysine-containing beads before ganglioside attachment. In the control experiment without beads interferon solution was incubated alone and diluted with corresponding amounts of 1 M NaCl and PBS. The numbers represent the reciprocal of the dilution of combined supernatants and washings showing a cytopathic effect of 50%.

L cells with PHA suggests involvement of carbohydrate-containing cell membrane constituents in interferon binding and/or action³. Our data show binding of interferon to gangliosides and inhibition of interferon action by exogenous gangliosides. They also demonstrate that inhibition by and binding to gangliosides is partially or completely reversed by PHA, in accord with our previous results³. Binding to gangliosides has been shown for certain toxins, like cholera toxin and tetanus toxin, for hormones like serotonin⁶, and for viruses like Sendai⁹. Our data suggest that binding to gangliosides might also play a role in interferon action *in vivo*.

Table 4 Inhibition of interferon activity by ganglioside-Sepharose beads

Beads used	Bead to cell ratio	Viral yield (PFU $\text{m}^{-1} \times 10^{-5}$)			
		No interferon added		Interferon added	
		I	II	I	II
None	—	1,200	740	5	3
Control beads	1:8	ND	340	ND	2
Ganglioside beads	1:8	ND	430	ND	170
Control beads	1:10	800	ND	5	2
Ganglioside beads	1:10	800	ND	200	170
Control beads	1:20	ND	ND	ND	2
Ganglioside beads	1:20	ND	ND	ND	32

Polylysine-Sepharose (control) or ganglioside-Sepharose beads prepared as described under Table 3 were suspended in 1 ml of MEM without serum and layered on to mouse L cells in 35 mm plastic Petri dishes (5×10^5 cells per dish). Mouse interferon prepared as described under Table 3 was added and the cells were incubated at 37°C for 24 h. After removal of medium (derivatised Sepharose beads remained tightly attached to the cells) the cells were infected with VSV at a multiplicity of infection of 0.1 and further incubated for 16 h at 37°C . Viral yield was measured directly by determination of plaque-forming units (PFU). I and II represent two different sets of experiments. ND, Not done.

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Evidence for ATP action on the cell surface

It seems that Mg^{2+} -ATPase (EC 3.6.1.3) and 5'-nucleotidase (EC 3.1.3.5) are prevalent plasma membrane marker enzymes in eukaryotic cells¹. Our studies on the 'sidedness' of plasma membrane macromolecules suggested that the two enzymes

function as ecto-enzymes², although the substrates presumably were not present on the cell surface. We assumed that the substrates were present, but obscured within the intercellular spaces and by rapid metabolism. The specificity of the ecto-ATPase is low but we suspected that translocated cytoplasmic ATP might be a primary substrate.

We have recently obtained evidence in tissue cultures for apparent ATP translocation from the cytoplasm into the medium (unpublished data). Reports by McIlwain *et al.* that adenosine, and to a lesser extent adenylates, were released from electrically stimulated brain slices support this possibility^{3–5}. Pull and McIlwain had speculated⁴ that ATP was the source of the released adenosine. We postulated that the translocation of cytoplasmic ATP might be part of a common physiological phenomenon. During a study of the effects of ATP application to the membrane surface I have now found that the addition of ATP to monolayer cultures of mammalian cells produces a biphasic change in membrane permeability. *In situ* this event may be initiated by the translocation of cytoplasmic ATP and terminated by ecto-nucleotidophosphoesterhydrolases.

Changes in membrane permeability were measured by determining the extrusion of isotopic ions from pulse labelled monolayer tissue cultures. I shall discuss the permeation of isotopic species because measurements of net fluxes have not been made yet. The basic methodology was briefly as follows. Established tissue culture cell lines were grown in 250 ml Falcon flasks in the appropriate medium. When the cultures reached confluency they were exposed for 2 h to 5 ml of medium containing ^{32}P , $^{42}\text{K}^+$ or other isotopic compounds. After removal of the isotopic medium by several brief rinses, the cultures were superfused with 4 ml of serum-free medium at 37°C in an air/ CO_2 atmosphere (95/5, v/v). During incubation the flasks were rotated horizontally once every 150 s for 6 s at 50 r.p.m. to provide stirring action. Extrusion of isotopic species was measured by sampling the medium at intervals and by scintillation counting. Protein content of the cultures was determined in NaOH digests according to Lowry *et al.*⁶.

Using ^{32}P pulse labelled cultures of mouse fibroblasts (L929), HeLa cells (human choriocarcinoma) or KB cells (human carcinoma of the nasopharynx) I found that ^{32}P efflux into the medium increased significantly when ATP reached about $5 \times 10^{-4}\text{ M}$. In monolayer cultures of neonatal Syrian hamster astrocytes, the effect of ATP is particularly evident and typical dose responses are illustrated in Fig. 1. When the superfusate

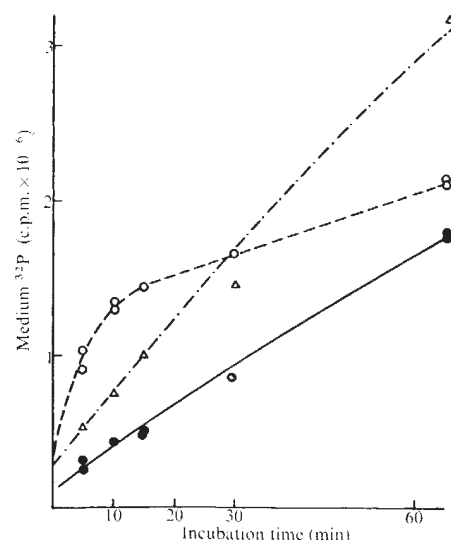


Fig. 1 Effect of medium ATP concentration on ^{32}P extrusion of pulse labelled NN astrocyte cultures. Cultures labelled with ^{32}P for 2 h, rinsed three times and superfused with Hanks' minimum essential medium containing ATP as indicated. Curves show cumulative ^{32}P in medium: Δ , $5 \times 10^{-3}\text{ M}$; $\circ$, $1.2 \times 10^{-4}\text{ M}$; $\bullet$, control.

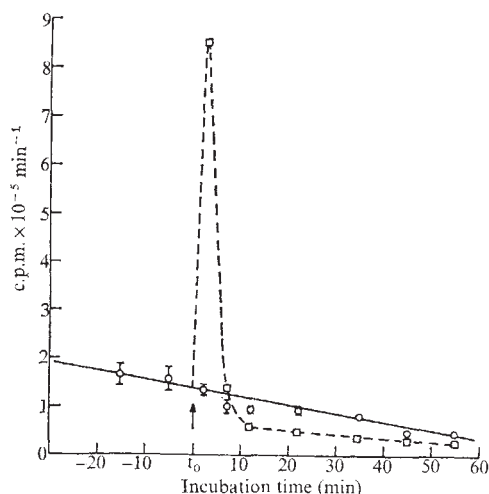


Fig. 2 Rate of extrusion of $^{42}\text{K}^+$ from NN astrocyte cultures after 6.25×10^{-5} M ATP in the medium at t_0 . Cultures labelled for 2 h with 5 mM ^{42}KCl , rinsed and superfused with Hanks' minimum essential medium. Average of duplicate experiments.

ATP concentration was 5 mM, ^{32}P extrusion into (and accumulation in) the superfusate proceeded at an accelerated steady rate. At lower concentrations initial efflux was even faster but soon decreased below the rates of the controls.

Testing of other compounds showed that a signal of the sort given by ATP could only be elicited with nucleotide triphosphates, and UTP, GTP and ITP were about equally effective. This was reminiscent of the ecto-ATPases with a similar lack of specificity towards nucleosidetriphosphates². Tests with other ATP metabolites revealed that adenosine (but not uridine, guanosine or inosine) had an effect opposite to that of ATP. Adenosine decreased the extrusion rate of ^{32}P from labelled cultures and inhibited ATP-induced acceleration of ^{32}P extrusion at 10^{-5} M; the effect seems to apply specifically to the transfer of ^{32}P .

A typical experiment with $^{42}\text{K}^+$ pulse labelled NN astrocytes is illustrated in Fig. 2. After measuring the extrusion rate of $^{42}\text{K}^+$ for 30 min, 6.2×10^{-5} M ATP was applied at t_0 , resulting in an immediate fivefold increase in $^{42}\text{K}^+$ efflux rate. Shortly thereafter apparent flux decreased below control levels, producing a biphasic signal in response to ATP application. A similar, apparently excitatory-inhibitory, response to ATP was observed by Harary and Farley in cultured beating rat heart cells⁷. At appropriate doses the effect of ATP on pulse labelled cells was observed in the N-18 neuroblastoma, HeLa cells, KB cells, glioblastoma GL-26 and L-929 fibroblasts. Different cell lines had significantly different dose responses to added ATP. The NN astrocytes responded to 5×10^{-7} M ATP, the N-18 neuroblastoma with not less than 5×10^{-4} M (Fig. 3) and sea urchin eggs, human erythrocytes and a human oligodendroglioma failed to respond.

The effect of ATP on ion permeation was temperature dependent. In the absence of Ca^{2+} no effect was obtained or it was markedly diminished. In the NN astrocytes 2 mM Ca^{2+} was optimal and higher calcium concentrations inhibited the signal produced by ATP. Addition of 10% foetal calf serum to the superfusate did not inhibit the ATP effect. Attempts to demonstrate the ATP effect in cultures pulse labelled with $^{22}\text{Na}^+$ or $^{36}\text{Cl}^-$ were not successful because these monolayer cultures are too clumsy a tool for studies which might involve time constants of the orders found in neurochemical transmission. Mostly using NN astrocyte cultures I have found that the ATP signal could not be elicited by acetylcholine, noradrenaline, caffeine, metrazol, inorganic pyrophosphate, phosphocreatin, 3', 5' cyclic AMP or strychnine. The following potential inhibitors proved inactive: ouabain, N-ethylmaleimide, colchicine, avidin, *p*-hydroxymercuribenzoate, ethylmorphine and theophylline. Oligomycin at 10 μg

ml^{-1} inhibited ecto-ATPase activity by about 50%. The same concentration partially inhibited the ATP signal in some cultures, but it also effected a moderate increase in extrusion rates by itself. This again suggests an interrelationship between ecto-ATPase and the ATP-induced increase in permeation rates.

Earlier observations may support the conclusion that these data reveal a physiological response. The apparent translocation of ATP from the cytoplasm into the environment has been observed under other circumstances. Abood *et al.*⁸ found a considerable increase in ^{32}P efflux from labelled frog spinal root nerves and sartorius muscle during electrical excitation. They concluded that the outflux of acid-soluble phosphates, particularly $^{32}\text{P}_i$ and ATP^{32}P , was somehow related to physico-chemical changes of membrane depolarisation. In analogous experiments Silinsky and Hubbard⁹ detected ATP release from phrenic nerve-hemidiaphragm by the bioluminescence assay. Boyd and Forrester¹⁰ observed release of ATP from frog skeletal muscle *in vitro* and it was also shown that ATP was released from exercising human forearm muscle^{11,12}. An alternative explanation of the occurrence of extracellular ATP was advanced by Agren *et al.*^{13,14} who observed apparent extracellular ATP synthesis by yeast cells and by various normal and neoplastic tissue cultures. The release of purine nucleotides (by implication the translocation of ATP) is one of the principal elements of a purinergic autonomic nervous system which has been reviewed in detail by Burnstock¹⁵.

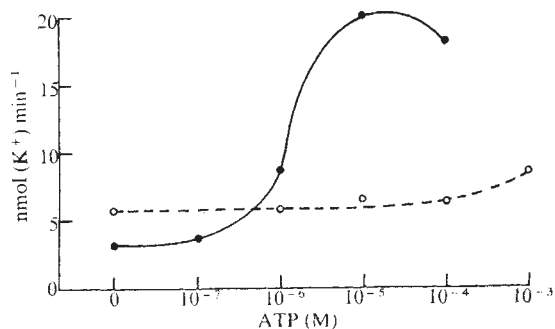


Fig. 3 Rates of extrusion of ^{42}K from pulse labelled NN astrocyte (●) and N-18 mouse neuroblastoma (○) cultures as a function of ATP concentrations at t_0 . Rates as efflux of ^{42}K min^{-1} during the first 5 min after ATP addition.

The significance of my observations is that they offer an explanation for the presence of some enzymes on the surface of the cell. If ATP is translocated following a pertinent stimulus, it might impinge on the membranes of adjacent cells, effecting an increase in permeability. In addition to the likely efflux of K^+ , the respondent cell might extrude some of its cytoplasmic ATP and thereby initiate the spreading or cascading transmission of a signal. Ecto-ATPase and ecto-5'-nucleotidase would then serve to limit or terminate the phase of increased permeability. In the microenvironment a change in permeability or excitability threshold might not be affected by the action of free ATP on the cell surface but by a membrane-bound species. Conceivably, the entire process (presumably of altering the plasma membrane structure briefly) requires a phosphorylation reaction on the outer aspect of the membrane. One biocatalyst for this reaction might seem to be an ecto-ATPase.

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Relationship of α -adrenergic receptors in rat pineal gland to drug-induced stimulation of phospholipid metabolism

NORADRENALINE produces increased uptake of $^{32}\text{P}_i$ into acidic phospholipids in the nervous system^{1–4}, and neurotransmitters and other stimuli do so in various tissues^{5–7}. The rat pineal gland responds to noradrenaline in similar fashion^{8–10}. We have shown already that the mechanism by which noradrenaline stimulates phospholipid metabolism in the pineal gland does not depend on protein synthesis and does not involve either β -adrenergic receptors or cyclic AMP¹⁰. We also observed that the β -adrenergic receptor blocking agent propranolol not only failed to counteract the influence of noradrenaline, but itself markedly enhanced the incorporation of $^{32}\text{P}_i$ into phospholipids to give a labelling pattern distinct from that produced by noradrenaline^{10,11}. We concluded that this action of propranolol was due to its local anaesthetic or membrane perturbing property, since sotalol, a β -adrenergic receptor blocking agent without these other effects¹², had no influence on phospholipid metabolism. In addition, a series of local anaesthetics yield effects comparable with those of propranolol¹³.

We now have clear evidence for two mechanisms for the stimulation of phospholipid metabolism by pharmacological agents *in vitro*. Using the rat pineal gland, we found that the effects of noradrenaline and the α -adrenergic agonist phenylephrine on pineal phospholipid metabolism are counteracted by α -adrenergic blocking agents, but that those of propranolol are unaffected.

Pineal glands were removed from ether-anaesthetised, female Charles River rats (150–200 g) and stored in ice-cold incubation medium until used. Individual glands were incubated free-floating in 100 μl of either modified Puck's N-16 medium (Grand Island Biological Co.) or Krebs-Ringer bicarbonate glucose medium containing 10 μCi of $^{32}\text{P}_i$ (New England Nuclear Corp.) and the desired amounts of drug. After incubation the glands were rinsed in saline and lipids were extracted with chloroform-methanol. Phospholipids were separated by two-dimensional thin-layer chromatography of the washed total lipid extract on Silica Gel H plates (Analtech). The areas which have been shown to correspond to the individual phospholipids under examination were scraped from the plates and counted^{10,11}.

Addition of L-noradrenaline (0.01 mM) increased incorporation of $^{32}\text{P}_i$ into phosphatidylinositol and phosphatidylglycerol by 200–300%, but had no effect on phosphatidylcholine labelling (Table 1). Similar stimulation was obtained with the α -adrenergic agonist phenylephrine (0.01 mM). Neither of the α -adrenergic blocking agents used, phenoxybenzamine (Dibenzyl; Smith, Kline and French) and phentolamine (Regitine; CIBA) at 0.02 mM increased labelling

Table 1 Inhibition of adrenaline- or phenylephrine-induced stimulation of pineal phospholipid metabolism by α -adrenergic receptor blocking agents

Additions	Incorporation of $^{32}\text{P}_i$ into		
	Phosphatidyl-inositol	Phosphatidyl-glycerol	Phosphatidyl-choline
(pmol per pineal gland)			
Noradrenaline	26.4 $\pm$ 3.5	1.1 $\pm$ 0.1†	16.8 $\pm$ 4.0
+ Phentolamine			
Noradrenaline	22.4 $\pm$ 4.0	0.8 $\pm$ 0.2	12.9 $\pm$ 1.6
+ Phenoxybenzamine			
Noradrenaline	66.3 $\pm$ 5.9*	2.0 $\pm$ 0.1*	16.9 $\pm$ 1.6
+ Phenylephrine			
Phentolamine	23.5 $\pm$ 2.9	0.9 $\pm$ 0.1	16.7 $\pm$ 4.6
+ Phenylephrine			
Phenoxybenzamine	20.6 $\pm$ 4.0	0.9 $\pm$ 0.1	11.7 $\pm$ 4.2
+ Phenylephrine			
Phenylephrine	54.2 $\pm$ 2.8*	1.7 $\pm$ 0.1*	15.3 $\pm$ 1.9
+ Phentolamine			
Phentolamine	15.1 $\pm$ 1.9	1.1 $\pm$ 0.1†	14.2 $\pm$ 1.2
+ Phenoxybenzamine			
Phenoxybenzamine	20.1 $\pm$ 2.8	1.2 $\pm$ 0.1†	15.5 $\pm$ 2.1
+ None			
None	18.6 $\pm$ 1.6	0.8 $\pm$ 0.1	15.9 $\pm$ 3.1

Pineal glands were incubated in modified Puck's medium for 1 h in the presence of the agonists (0.01 mM), the blocking agents (0.02 mM) or both drugs together. Each value represents the mean $\pm$ s. e. of the mean of at least four pineal glands. Differences from control means (no additions).

* $P < 0.001$.

† $P < 0.05$.

of phosphatidylinositol, although some stimulation by phentolamine occurred with much larger concentrations of drug. Combinations of agonist and antagonist virtually abolished the response to agonist alone. Both α -receptor blocking drugs prevented the enhanced $^{32}\text{P}_i$ incorporation into phosphatidylinositol and phosphatidylglycerol elicited by either noradrenaline or phenylephrine. These observations strongly indicate the presence of α -adrenergic receptors in rat pineal gland and their involvement in mediating the action of both phenylephrine and noradrenaline, the physiological pineal neurotransmitter, on pineal phospholipid metabolism.

Our finding that propranolol strongly stimulated $^{32}\text{P}_i$ incorporation into lipids¹⁰ prompted us to attempt to discriminate between the modes of action of noradrenaline and propranolol. Such a distinction was suggested by different labelling patterns of phospholipids produced by these agents. The differences include stimulation of cytidine diphosphate-diglyceride labelling and increased incorporation of isotope into phosphatidylglycerol and phosphatidic acid in the presence of propranolol compared with noradrenaline. We therefore investigated whether an α -adrenergic receptor blocker affected propranolol-induced stimulation of pineal phospholipid metabolism. Although it prevented the effects of noradrenaline or phenylephrine, phenoxybenzamine did not reduce the effects of propranolol (Table 2). The same resistance of propranolol-induced stimulation of pineal phospholipid metabolism to α -adrenergic receptor blockade was observed in experiments with Puck's medium and smaller concentrations of phenoxybenzamine or phentolamine (0.02 mM).

There are already indications that α -adrenergic blocking agents inhibit adrenaline-induced nonspecific stimulation of phospholipid metabolism, obtained with liver slices¹⁴ and fat cell suspensions¹⁵. Robison *et al.* speculated that α -adrenergic receptors mediate the effects of catecholamines on phospholipid metabolism¹⁶. Later work has shown the ability of phenoxybenzamine or phentolamine to block enhanced phosphatidylinositol labelling from $^{32}\text{P}_i$, produced by adrenaline, noradrenaline or phenylephrine, in slices of parotid gland^{17,18} and vas deferens¹⁹ as well as in brain *in vivo*⁴.

The findings reported here are consistent with these observations. We have demonstrated that the rat pineal gland

Table 2 Resistance of propranolol-induced stimulation of pineal phospholipid metabolism to α -adrenergic blockade

Additions	Concentration (mM)	Phosphatidyl-inositol	Incorporation of $^{32}\text{P}_i$ into		Phosphatidic acid	Phosphatidyl-choline
			Phosphatidyl-glycerol (pmol per pineal gland)	CDP-diglyceride		
Propranolol	0.03					
+						
Phenoxybenzamine	0.06	14.1 $\pm$ 0.8*	3.8 $\pm$ 0.4*	—	3.9 $\pm$ 0.5†	8.0 $\pm$ 0.4
Propranolol	0.03	15.0 $\pm$ 0.9†	3.4 $\pm$ 0.3*	—	4.4 $\pm$ 0.3†	8.3 $\pm$ 0.8
Propranolol	0.10					
+						
Phenoxybenzamine	0.06	18.8 $\pm$ 1.2*	8.4 $\pm$ 0.3*	3.6 $\pm$ 0.5*	7.2 $\pm$ 0.2*	8.7 $\pm$ 1.6
Propranolol	0.10	16.6 $\pm$ 2.4†	7.5 $\pm$ 0.9*	5.0 $\pm$ 0.9†	6.0 $\pm$ 0.8†	7.2 $\pm$ 0.9
Phenoxybenzamine	0.06	8.2 $\pm$ 0.8	0.4 $\pm$ 0.0	0.2 $\pm$ 0.1	3.2 $\pm$ 0.2	10.4 $\pm$ 0.6‡
None		8.5 $\pm$ 0.4	0.5 $\pm$ 0.1	0.3 $\pm$ 0.0	2.5 $\pm$ 0.2	6.6 $\pm$ 0.5

Pineal glands were incubated in Krebs-Ringer bicarbonate buffer for 1 h in the presence of DL-propranolol, phenoxybenzamine or both agents together. Each value represents the mean $\pm$ s. e. of the mean of four pineal glands.

Differences from control means (no additions). * P < 0.001; † P < 0.005; ‡ P < 0.02.

possesses α -adrenergic receptors which mediate the stimulatory effects of noradrenaline on pineal phospholipid metabolism. Earlier the suggestion was made that in the pineal gland "the response to β -adrenergic stimulation can be influenced by an α -adrenergic mechanism", since phentolamine enhanced the effect of noradrenaline on serotonin N-acetyl transferase activity²⁰. Until now only β -adrenergic receptors have been definitely shown to be present in the pineal gland²¹.

It has been suggested that α and β -adrenergic receptors act through a common mechanism, involving opposite effects on the intracellular level of cyclic AMP¹⁶. The authors of this hypothesis also cite instances, however, in which α -adrenergic receptor-mediated phenomena are not accompanied by a decrease in cyclic AMP. The pineal gland provides a further instance of stimulation of α -adrenergic receptors which does not depress cyclic AMP, since noradrenaline increases the level of this nucleotide²¹.

In rat parotid slices α -adrenergic receptors mediate the release of intracellular potassium by adrenaline, a process which requires Ca^{2+} and energy²² and which can similarly occur without changes in the cyclic AMP level of the cell. These observations suggest that the physiologically significant aspect of the stimulation of the acidic phospholipid metabolism brought about by catecholamines involves a relationship between the α -adrenergic receptor and ion movements. The pineal gland may be a suitable model system for the study of these interrelationships.

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Octopamine receptors on *Aplysia* neurones mediate hyperpolarisation by increasing membrane conductance

OCTOPAMINE is a phenylethylamine synthesised by β -hydroxylation of tyramine and differs from noradrenaline in lacking one hydroxyl group on the phenyl ring. Although *Aplysia* nervous tissue, like that of most molluscs, contains little or no noradrenaline^{1,2}, it has between 0.20 and 1.85 pmol octopamine per mg of tissue in the various ganglia. Individual identified neurones also contain detectable octopamine and in neurone R14 it reaches 1.5×10^{-4} M (ref. 2). Although octopamine-activated adenylyl cyclases have been found in *Aplysia* nervous tissue³ and insects^{4,5}, there has been no electrophysiological demonstration of specific octopamine receptors. We now report receptors in the nervous system of *Aplysia* that are most sensitive to octopamine and mediate a hyperpolarising conductance-increase response. This provides further evidence that octopamine can function as a neurotransmitter.

Abdominal, cerebral, pleural and pedal ganglia of *Aplysia californica* or *Aplysia dactylomela* were removed and pinned to Sylgard (Dow Corning) in a lucite chamber. The connective tissue capsule was slit with a razor blade to expose the cell bodies. Neurones were penetrated with a double barrelled glass micropipette filled with 2 M potassium acetate and recording was performed as before⁶. Constant current pulses and/or current were applied through one barrel of the electrode as needed, while recording was made through the other. The

ganglia were constantly perfused with high Mg^{2+} (150 mM) seawater, which blocks transmitter release from nerve terminals. Consequently, responses to iontophoretic drug application may be considered to be direct effects.

Octopamine and related compounds were dissolved in distilled water at concentrations of 1 M (pH 3–4) and each compound was placed in one barrel of a five barrelled iontophoretic electrode which was bumped at the tip to give a resistance of 2–5 $M\Omega$ for each barrel. The controller for the iontophoretic current was designed to pass a constant charge between 0 and 1,000 nC (J. Willis, in preparation). Thus the duration of the pulse was varied automatically to ensure that the same total charge was passed from each barrel, making possible a meaningful comparison of the response of one cell to several putative transmitters. When an octopamine response was found, the sensitivity of that site was tested with all other drugs in the iontophoretic electrode.

Responses to octopamine were recorded in cells from all ganglia studied but were obtained in only a small percentage of neurones. They were most common in cerebral ganglia, which also contain the most octopamine³. In all cases receptors for octopamine, like those for dopamine⁷, were not on the soma but rather in the neuropile. This may have led to an underestimation of the percentage of neurones containing octopamine receptors since it was necessary to search blindly for sensitive areas in the neuropile.

Figure 1 illustrates the response of a neurone in the cerebral ganglion to octopamine. A pulse of 1,000 nC octopamine resulted in a slow hyperpolarisation peaking at about 12 S. Pulses of 2,000 nC noradrenaline and phenylethanolamine, which differ from octopamine in having one additional and one less hydroxyl groups respectively, gave small responses. Dopamine was ineffective in exciting this receptor.

Figure 2 illustrates another experiment in which a pulse of 500 nC octopamine gave a 5 mV hyperpolarisation (a). When constant pulses (10^{-10} A) were applied (b), the amplitude of the voltage deflection during the response was reduced. This reduction was not a result of nonlinear current-voltage relationships (not illustrated), and thus reflects an increased conductance to some ion, such as K^+ or Cl^- , which has an equilibrium potential more negative than the resting potential. In (c) the ganglion was perfused with seawater in which all Cl^- was replaced with acetate, which is relatively impermeable. In such a solution the equilibrium potential for Cl^- shifts and becomes depolarising, as is the case for Cl^- conductance responses to acetylcholine⁸. This response did not change, how-

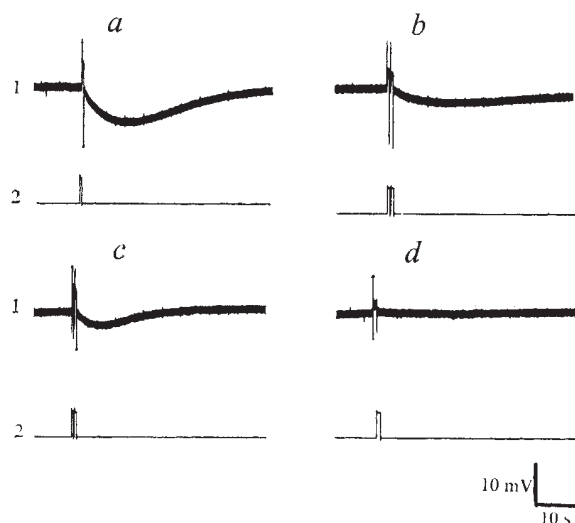


Fig. 1 Response of an unidentified neurone in the cerebral ganglion to octopamine (a), phenylethanolamine (b), noradrenaline (c) and dopamine (d). Trace 2 shows the duration of the iontophoretic pulse. Each pulse passed 1,000 nC. Double pulses were given for phenylethanolamine and noradrenaline.

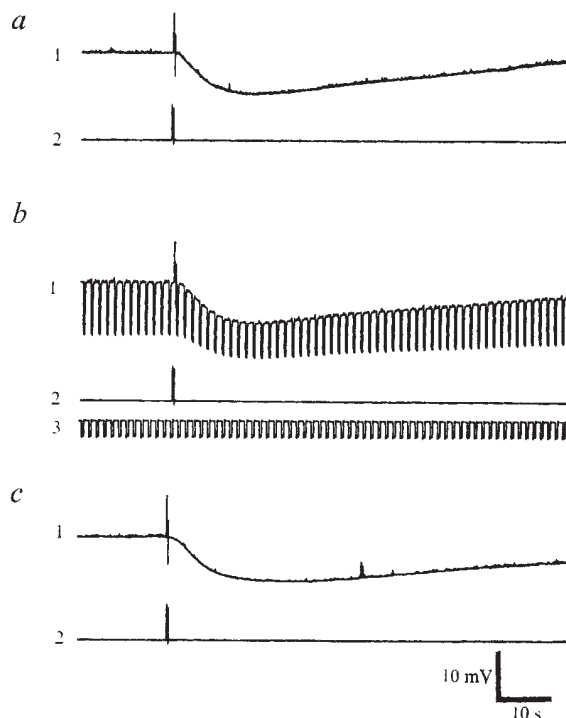


Fig. 2 Responses to 500 nC octopamine from an experiment on another unidentified neurone in the cerebral ganglion (a). For trace (b) constant current pulses (10^{-10} A) were applied during the application of octopamine. The smaller amplitude of the voltage deflection during the responses indicates a conductance increase since the neurone had a linear current-voltage relationship over this range of potential. For trace (c) the ganglion was perfused with Cl^- -free (acetate) seawater. Membrane potential was adjusted to control levels by applied depolarising current. The fact that the response is as large as control indicates that it is not a result of a Cl^- conductance increase.

ever, and thus it is likely that it results from an increased K^+ conductance. It was not possible to reverse most of these responses by applied hyperpolarisation. This is probably because the receptors are all on distal processes in the neuropile. A similar difficulty has been reported in reversing K^+ conductance dopamine responses⁷.

The evidence for a neurotransmitter function for octopamine in *Aplysia* is threefold. (1) It is present in ganglia and is asymmetrically distributed in single, identified neurones. It is present in neurones that contain neither dopamine nor noradrenaline in concentrations as high as 10^{-4} M (ref. 2). (2) Octopamine receptors are located on a few neurones only and then only in the neuropile, where functional synapses occur. (3) The receptors have a marked specificity for octopamine. Most are also slightly sensitive to noradrenaline and phenylethanolamine, but to a much smaller degree. This suggests that the receptor has an absolute requirement for the β -hydroxyl group and as a result is insensitive to dopamine. Since there is no noradrenaline in *Aplysia*^{1,2}, the responses to it have no functional significance. Phenylethanolamine is present in *Aplysia* nervous tissue (J. Saavedra, M. Brownstein, D. O. C. and J. Axelrod, unpublished observations) and receptors specifically sensitive to phenylethanolamine are present (our unpublished observations). The sensitivity of the receptors illustrated here is so much greater for octopamine that it seems likely that this is their physiological function. Neurones of the land snail respond to octopamine as well as dopamine and noradrenaline, but none have clearly specific receptors for octopamine only⁹.

Octopamine is present in the nervous and innervated non-nervous tissues of mammals¹⁰ as well as various invertebrates^{9,11}. But, as octopamine can replace noradrenaline at its storage sites and be released on nerve stimulation^{12,13}, it has been considered to be a false neurotransmitter. Molinoff and Axelrod¹⁴ proposed

that, since octopamine is a normal constituent of rat sympathetic nerves, it might normally be released together with noradrenaline. The combination of the demonstration of octopamine in neurones not containing other phenylethylamines and receptors very specific for octopamine argues strongly for a neurotransmitter function of octopamine in *Aplysia* which is independent of any other structurally related compound.

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Sustained oscillations of acetylcholine during nerve stimulation

THE neurotransmitter acetylcholine (ACh) is synthesised and stored in nerve terminals. Its turnover, rather slow under resting conditions, is greatly accelerated by nervous activity. When action potentials reach the terminals they trigger the release of large amounts of transmitter into the synaptic cleft where ACh can act on postsynaptic receptors and become a substrate for cholinesterase. Then choline and in some cases¹ acetate moieties are taken up by the nerve endings and incorporated into new ACh which, in turn, can be rapidly re-used. To investigate the turnover of ACh during activity, we have measured acetylcholine at short time intervals after the exposure of the nerve to electrical stimulation at different frequencies. We report here that the concentration of ACh oscillates following the onset of stimulation, and that the period of oscillation is related to the stimulation frequency. The experiments have been performed using the electric organ of *Torpedo*, a purely cholinergic tissue, which is very homogeneous and rich in ACh^{2–4}.

Torpedo marmorata fishes were received alive from the Station de Biologie Marine, Arcachon, France. Most experiments were done on isolated prisms, which are columns of electroplaques representing the functional unit of the organ³. After careful dissection, the prisms were allowed to recover for 1–2 h in an elasmobranch saline medium which maintains the functional and structural properties of the tissue⁵. The prisms were submitted to electric field stimulation for a given time and then rapidly quenched, by one of the following methods. (a) Tissue was plunged into liquid nitrogen, pulverised and allowed to thaw in trichloroacetic acid (TCA) which was then extracted with ether, and the ACh determined using the

frog rectus muscle bioassay. (b) Saline elasmobranch medium acidified to pH 4 with HCl at 100°C was used. The results obtained with these two methods were identical. The time lag between the end of stimulation and immersion in liquid nitrogen or boiling acidified medium did not exceed 1–2 s.

Figure 1a shows the typical changes in ACh content which occur when the tissue is stimulated. At rest (time of stimulation = 0) there was a rather large dispersion of ACh values, perhaps because the prisms were at different starting conditions. During the first 2 s of stimulation, ACh content decreased. After this the first peak was seen, which in all experiments looked sharp and exceeded the mean resting content. The second peak was always lower; at this stage there was again a large dispersion of values. But 20 s from the onset of stimulation,

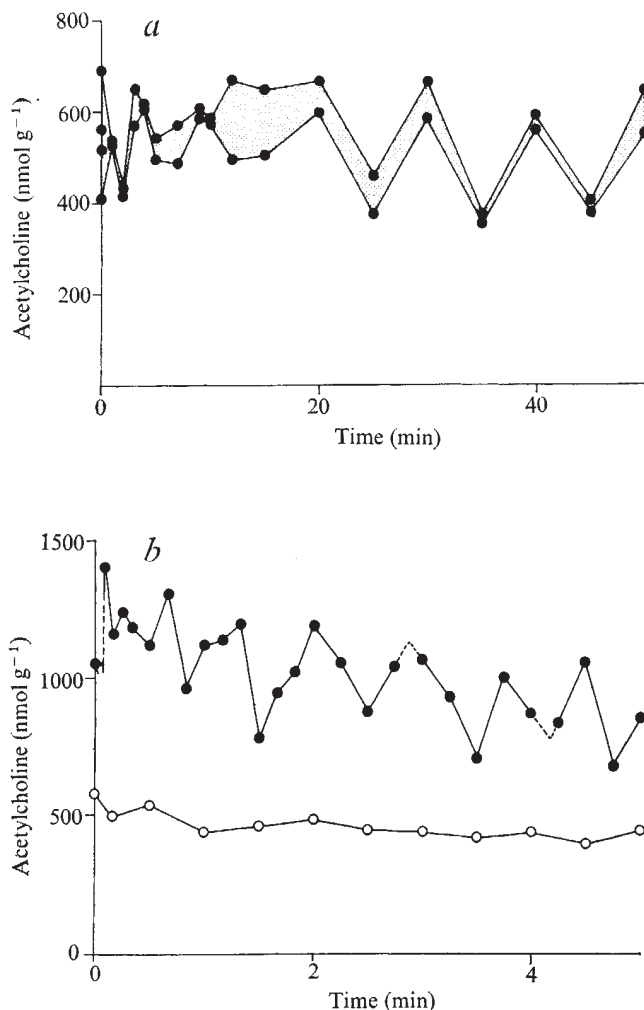


Fig. 1 Dynamic pattern of ACh changes during stimulation. ACh is expressed as nmol per g wet tissue. *a*, During stimulation at 20 Hz, the period of oscillation is too close to the frequency of sampling to permit a description of the form of the oscillations (results from two experiments on the same *Torpedo*). *b*, Stimulation at 10 Hz. The bound or vesicular compartment of ACh (○) did not follow the large oscillations of total ACh (●), therefore the changes are largely in the "free" compartment.

the amount of ACh started to show oscillations with a regular period of about 10 s for a stimulation frequency of 20 Hz.

In Fig. 1b the tissue was given a stimulation of 10 s⁻¹. After the initial changes (the first fall, missed here, was found at 2 s in another experiment) ACh content oscillated with a period of about 42 s. In this experiment, bound or vesicular ACh was also measured in prisms by immediately homogenising after stimulation and then treating with TCA. In this

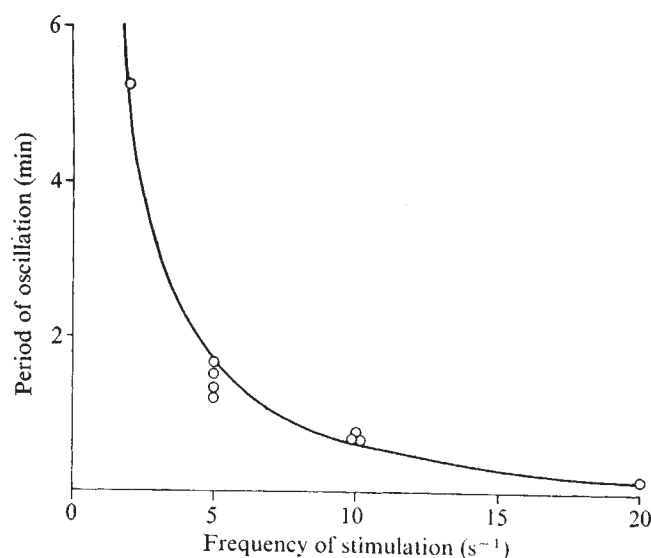


Fig. 2 Period of the ACh oscillations as a function of stimulation frequency. The line is an hyperbola calculated for the best approximation to experimental values.

case, the ACh remaining in the extract is regarded as ACh bound in a compartment associated with synaptic vesicles^{4,6} whereas ACh hydrolysed during homogenisation (about half of the total amount) is "free" or "available" ACh^{5,7}. Fig. 1b shows that bound ACh did not oscillate during stimulation. Clearly the large variations in the total ACh concerned only the available pool of transmitter. If stimulation is continued, however, a decrease in the vesicular ACh also will occur^{5,7,8}.

The variations in ACh content followed the same general pattern at other stimulation frequencies. The period of oscillation approximated a hyperbolic function of the stimulation frequency (Fig. 2). The delay of the first peak obeyed a slightly different relationship, reaching a limit at high stimulation frequencies. At the lower frequencies the shape of the oscillation was far from a sinusoidal wave; the peaks were separated by flat intervals and their rising slope looked smaller than their fall. In a few cases, the electric tissue was also stimulated *in vivo* through the nerves, the animal being slightly anaesthetised⁵. The results obtained under these conditions were the same as those from excised prisms.

Thus we found that, when driven from the resting to the active state, the electrogenic tissue exhibits a remarkable dynamic pattern. After a somewhat complicated initial phase, the ACh content follows undamped oscillations which are a function of the stimulation frequency. The dispersion of ACh values, rather high at the beginning, decreases during the oscillations. This behaviour could hardly be explained on the basis of a first order kinetic system, even if the conditions were far from equilibrium⁹. On the other hand, sustained oscillations have been predicted and observed in more complicated systems coupled with a flow of matter or energy¹⁰⁻¹². The oscillation arises at a key reaction, perhaps as a result of substrate inhibition or product activation of the reaction, although it can be caused in many other ways. For example, diffusion velocities to or through membranes can be important in such phenomena^{13,14}. The result is that, when the rates of provision of substrate and removal of product fall between well defined limits, the system follows undamped oscillations, whose period is a function of the rate of provision of substrate. The flux through these systems is subject to sudden variations between two or more steady states, and the initial transition does not occur at the same substrate concentration as the reverse transition.

The resting turnover of ACh in this tissue (ref. 15 and unpublished observations) is about 10^4 times lower than the maximum speed of net synthesis measured in the present

experiments. We think that the mechanisms underlying ACh oscillations are more than a curiosity. They are probably the result of highly regulated metabolic loops and might be a general property of pathways which, when passing from rest to activity, require accelerations of several orders of magnitude.

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Evidence for a direct effect of LRF and TRF on single unit activity in the rostral hypothalamus

MANY of the hormones produced by the pituitary and other endocrine glands influence the electrical activity of the brain (for recent references see Dyer¹). There is now a growing awareness that some of the hypothalamic releasing factors, which regulate the secretion of hormones from the anterior pituitary gland, may themselves have a similar influence upon the central nervous system²⁻⁴. The amino acid sequences for both thyrotrophin releasing factor [TRF; (pyro) Glu-His-Pro (NH₂)]^{5,6} and luteinising hormone releasing factor [LRF; (pyro) Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly (NH₂)]⁷ have been fully established and these polypeptides, produced synthetically and of high specific activity, are readily available. The experiments to be described were undertaken to establish whether LRF and/or TRF have a direct effect upon the electrical activity of neurones in the forebrain. Action potentials were recorded from single cells in the hypothalamus and cerebral cortex and LRF and TRF applied to them by microiontophoresis. The sensitivity of some of the neurones to oxytocin (a biologically active peptide of similar size) was also tested.

Female rats were anaesthetised with urethane and prepared for extracellular recording of single unit activity⁸. Action potentials were recorded through glass micropipettes—filled

with 4.0 M sodium chloride—with a tip resistance of 5–15 M Ω , which constituted the centre barrel of multi-barrelled electrodes. These were made from glass electrode tubing (H15/10, Jencons) using established techniques and filled with 2.0 M sodium chloride, 0.01 M oxytocin (purified from natural material by Dr B. T. Pickering), 0.01 M LRF (Beckman Ltd and ICI Ltd) and 0.03 M TRF (Beckman Ltd). The overall tip diameter of the electrode was between 1.5 and 3.0 μ m and the tip resistance for each pipette containing drugs was in excess of 25 M Ω . The drugs were retained within the electrodes by currents not exceeding 5 nA and ejected with currents of up to 300 nA, but usually in the range 5–30 nA. (We have not attempted to express the amount of releasing factor applied in terms of mol s⁻¹ since the conversion from nA requires many assumptions⁹ and may produce a very misleading figure.) The polypeptides were only considered to affect the firing rate of a neurone when repeated applications caused at least a 20% change in the discharge rate of the cell over the test period. Routine applications were made for 40 s but some cells were tested for many minutes. Responses which could not be reproduced were excluded and where reproducible responses were obtained, their specificity was checked by application of oxytocin or Na⁺ at the same current. The apparatus and techniques used were similar to those previously described from this laboratory^{10,11}.

Thirty-six single units were fully tested and of these 12 were recorded in the cerebral cortex and 24 in the preoptic and anterior hypothalamic areas (PO/AH). Oxytocin, LRF and TRF did not affect any of the twelve neurones tested in the cerebral cortex. Oxytocin was also without effect on any of the 8 cells tested in PO/AH (Fig. 1). LRF inhibited the spontaneous discharge of 4 out of 12 cells, however, (Fig. 2) and TRF had a similar effect on 7 out of 17 cells (Figs 1 and 2). One cell was excited by TRF and the remaining cells were not affected. Some of the unresponsive cells were subsequently tested by iontophoresing the releasing factors for many minutes but none of the cells exhibited any change in their firing rate which could be attributed to the drug application (Fig. 3). Although not all neurones were fully tested with both LRF and TRF, several units responded to one releasing factor and not the other (Fig. 1). Two cells were inhibited by both LRF and TRF (Fig. 2).

These results show that the releasing factors for thyroid stimulating hormone and luteinising hormone have the

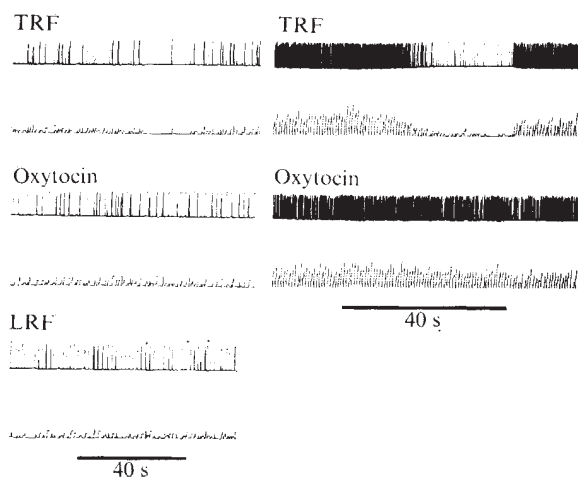


Fig. 1 The polygraph records on the left were obtained from a preoptic neurone which was inhibited by TRF (5 nA) but not LRF or oxytocin (8 nA). The two traces on the right show another unit inhibited by TRF and unaffected by oxytocin (both 7 nA). This second cell was not adequately tested with LRF. (Note, in this and the subsequent figures each action potential is represented by one excursion of the pen (top trace for each test) and an integrated record of this activity is shown in the lower trace. The test period is depicted by the black bar.)

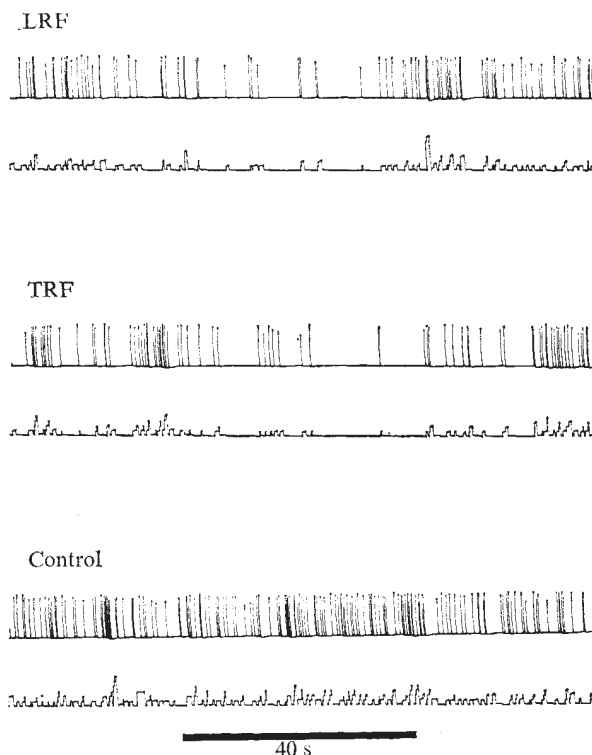


Fig. 2 Polygraph record showing inhibition of a single neurone in PO/AH by both LRF and TRF. The control was Na⁺ ejected at the same current (8 nA) as the releasing factors.

potential to modify the electrical activity of certain hypothalamic neurones. It is particularly interesting that these releasing factors did not affect all cells and did not influence the firing of any of the neurones tested in the cerebral cortex. It is also important that the firing rate of the responsive cells was neither increased nor diminished by microiontophoretic application of oxytocin at the same current, and that some cells responded to one releasing factor and not the other. Oxytocin has already been shown to excite antidromically identified neurosecretory cells in the paraventricular nucleus of rats and rabbits when applied extracellularly by microiontophoresis¹¹. The experiments also showed that the hormone did not affect non-neurosecretory cells in the paraventricular nucleus and we have now extended these findings to show that neurones in the rostral hypothalamus are unresponsive to oxytocin.

The units inhibited by application of releasing factor frequently responded when rather low ejection currents were used (for example about 10 nA) and one cell even showed a substantial reduction in firing rate when the retaining current on the TRF pipette was turned off. On the other hand, many of the unresponsive cells were tested, with the same electrode as responsive cells, at much higher currents and for longer periods of time. Our results show therefore that a discrete population of neurones in the rostral hypothalamus is inhibited by application of hypothalamic releasing factors.

If releasing factors are important in the control of hypothalamic function two possible routes of access to the neurones can be postulated. In the first place, the releasing factor may be secreted into the portal vessels from which they could be carried through the systemic circulation back to the hypothalamus. This is the conventional understanding of the so-called 'ultra-short loop' feedback system. An alternative mode of application could be trans-synaptic. That is, the releasing factors may be neurotransmitters secreted from branches of peptidergic neurones which do not terminate near the vessels of the primary plexus of the adenohypophyseal portal system. In fact, there is some evidence that fibres stained by immunofluorescence for LRF do not always pass to the

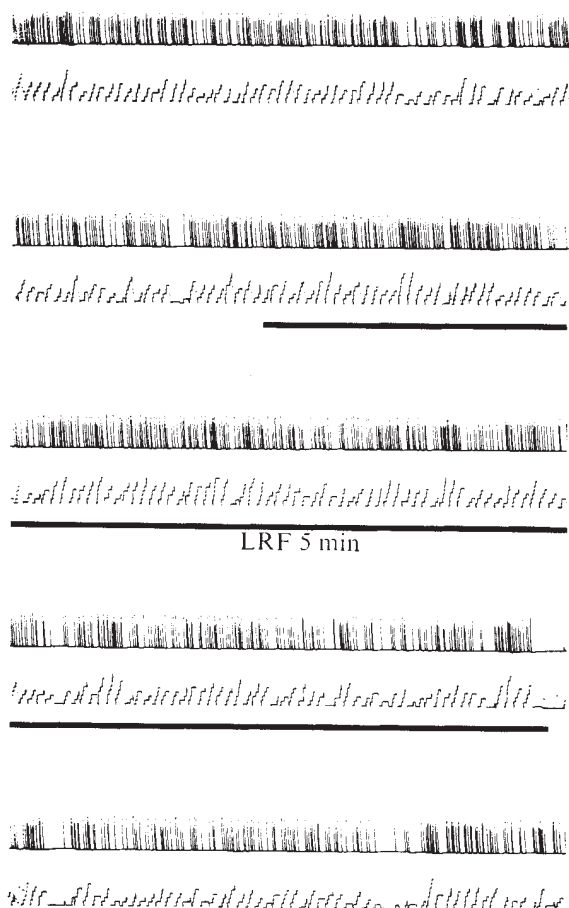


Fig. 3 Continuous polygraph record to show prolonged application of LRF (13 nA) on to a neurone which did not respond to the standard test period of 40 s. Note that even after 5 min the firing rate was unchanged.

median eminence. Such fibres have been described passing from the hypothalamus towards the mesencephalon and mammillary bodies as well as in the amygdala¹².

A neurotransmitter function for releasing factors, in addition to their classical role, could provide a possible mechanism for the coordination of different aspects of hypothalamic function. There is no theoretical reason why neurones which secrete releasing factors into the hypophyseal portal vessels may not have collateral branches which synapse on to other hypothalamic cells. In this way a single neurone or group of neurones in the hypothalamus could influence both the behaviour of the animal and secretion of its pituitary hormones.

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***In vitro* enhancement of hepatic microsomal biphenyl 2-hydroxylation by carcinogens**

MANY natural and synthetic chemicals in the environment may be carcinogenic, but it is clearly impracticable to assess the potential hazard from all such compounds by existing *in vivo* methods. Simple preliminary screening procedures are therefore urgently needed to identify the compounds most likely to be hazardous and allow detailed study of these suspected carcinogens. A series of fundamentally different test systems involving a range of biological materials will probably be necessary. As most carcinogens seem to act through active metabolites produced by the enzymes of mammalian endoplasmic reticulum¹, any test system probably requires the presence of these enzymes and the cofactor NADPH₂.

Assessing the damage to the DNA of growing bacteria which is produced by carcinogens in the presence of mammalian drug metabolising enzymes—whether *in vivo*² (host mediated assay) or *in vitro* using liver cell fractions (Ames test)³—seems to be the most successful approach so far. The estimation of changes inflicted on the endoplasmic reticulum, however, (such as the *in vitro* displacement of ribosomes (degranulation) of the liver endoplasmic reticulum by carcinogens in the presence of NADPH₂ (ref. 4)) and the *in vivo* effects of carcinogens on drug metabolising enzymes⁵ are promising alternatives.

Here we report that the *in vitro* incubation of some chemically dissimilar compounds which are carcinogenic to rat hepatic microsomes in the presence of NADPH, produces a selective increase in biphenyl 2-hydroxylation, but has no significant effect on biphenyl 4-hydroxylation. Non-carcinogens do not enhance either hydroxylase.

The incubation system was as follows: Test compounds (carcinogens and non-carcinogens) in either groundnut oil, or in 1.15% KCl plus 1% Tween 80, were added to hepatic microsomes in buffer solution prepared from male adult Wistar rats (final protein concentration of 2 mg ml⁻¹ in the presence of an NADPH-regenerating system of glucose-6-phosphate (25 mM), NADP (500 nmol), glucose-6-phosphate dehydrogenase (2 unit ml⁻¹) and magnesium sulphate (50 mM) and the system preincubated for 10 min at 37° C in a shaking water bath at 100 cycles min⁻¹. Biphenyl (13 mM in 1.15% KCl containing 1.5% Tween 80) was then added and the incubation continued for a further 5 min. The reaction was stopped by the addition of 1 ml of M HCl and the 2- and 4-hydroxybiphenyls thus formed were extracted and determined fluorimetrically⁶. In the controls, the test substance or biphenyl was either left out or added after the end of the reaction.

Preincubation of rat microsomes with a wide range of carcinogens such as 3,4-benz(a)pyrene, aflatoxin B₁, 2-acetamidofluorene and dimethylnitrosamine caused a significant enhancement of biphenyl 2-hydroxylation. In contrast, non-carcinogenic compounds, such as salicylate, phenobarbitone and 1,2,3,4-dibenz(a)pyrene, produced no significant effect on

the biphenyl 2-hydroxylase. Neither class of compound produced any significant changes in biphenyl 4-hydroxylation (Table 1). Interestingly, carbon tetrachloride, a hepatotoxic agent and a questionable carcinogen, produced only a slight enhancement of biphenyl 2-hydroxylation. It is apparent that the enhancement of the hepatic biphenyl 2-hydroxylase activity is not confined solely to hepatocarcinogens for β -naphthylamine, benz(a)pyrene and methylcholanthrene are all effective.

This is probably because our *in vitro* system has optimal conditions for the metabolism of most carcinogens, which produce their active metabolites through microsomal metabolism, a situation which clearly does not pertain *in vivo*. The effect on biphenyl 2-hydroxylase seems to be relatively specific because other drug metabolising enzymes, such as *p*-nitroanisole demethylase and aniline hydroxylase, were either unaffected or slightly inhibited following preincubation with carcinogens and non-carcinogens (F.McP., J.W.B., and D.V.P., unpublished). Similar results have been found using hamster liver microsomes. The possibility of the carcinogen provoking an allosteric conversion between the two hydroxylases seems unlikely in view of the apparent lack of correlation between changes invoked in biphenyl 2- and 4-hydroxylation.

Table 1 The effects of preincubation with carcinogenic compounds on the biphenyl 2-hydroxylase and biphenyl 4-hydroxylase of rat hepatic microsomes

Test compound*	Carcinogenicity	% Change in activity, relative to control values	
		Biphenyl 2-hydroxylase	Biphenyl 4-hydroxylase
3,4-Benz(a)pyrene	+	+252 $\pm$ 23†	-9 $\pm$ 6
22-Methylcholanthrene	+	+243 $\pm$ 19†	+8 $\pm$ 9
20-Methylcholanthrene	+	+125 $\pm$ 11†	-4 $\pm$ 7
2-Acetamidofluorene	+	+74 $\pm$ 9†	-9 $\pm$ 7
Dimethylnitrosoamine	+	+98 $\pm$ 11†	-18 $\pm$ 5
Aflatoxin B ₁	+	+87 $\pm$ 7†	+2 $\pm$ 6
Safrole	+	+82 $\pm$ 7†	-34 $\pm$ 6†
Piperonyl butoxide	?	+62 $\pm$ 5†	-31 $\pm$ 4†
Piperonyl sulphoxide	?	+62 $\pm$ 9†	-28 $\pm$ 7†
Isosafrole	?	+47 $\pm$ 3†	-34 $\pm$ 3†
<i>a</i> Naphthylamine	—	+17 $\pm$ 12	-4 $\pm$ 3
β Naphthylamine	+	+198 $\pm$ 14†	+3 $\pm$ 6
Carbon tetrachloride	?	+34 $\pm$ 8†	-39 $\pm$ 6†
Phenobarbital	—	+16 $\pm$ 9	-8 $\pm$ 6
Nikethamide	—	+10 $\pm$ 12	-12 $\pm$ 4
1,2,3,4-Dibenz(a)pyrene	—	+8 $\pm$ 9	+4 $\pm$ 7
Hexobarbital	—	+11 $\pm$ 6	+2 $\pm$ 5
Aniline	—	-12 $\pm$ 7	-5 $\pm$ 3
Salicylic acid	—	+3 $\pm$ 5	+4 $\pm$ 5
Piperidine	—	+7 $\pm$ 6	+4 $\pm$ 7
Piperanyl	—	0 $\pm$ 7	-3 $\pm$ 7
Eugenol	—	+3 $\pm$ 6	0 $\pm$ 8
EDTA	—	+2 $\pm$ 9	-37 $\pm$ 3†

Results $\pm$ s.e.m. for six animals

*All compounds were dissolved in 1.15% KCl and 1% Tween 80 and added to the incubation mixture to give a final concentration of 5×10^{-4} M except those in italics which were added as a solution in groundnut oil (Saladin) (0.5 mg in 0.5 ml) which also gives an approximate concentration of 5×10^{-4} M. Preincubation in each case was for 10 min.

†Indicates test results significantly different from control values ($P < 0.05$).

Our observations may be the result of a direct effect of the carcinogen itself or may be mediated by the formation of an active metabolite. It is generally accepted that most carcinogens produce their *in vivo* effects through their metabolism to an active electrophilic or free radical intermediate⁷⁻¹⁰, probably effected by the NADPH-cytochrome P-450-dependent microsomal hydroxylase system¹. Inhibitors of cytochrome P-450 could not be used directly to establish whether formation of an active metabolite from the carcinogen was a prerequisite for the enhancement of biphenyl 2-hydroxylase because they would also interfere with biphenyl metabolism.

Evidence that these carcinogens require metabolism before producing their effects, however, was obtained by examining the cofactor requirements and the incubation time dependency of the increased biphenyl 2-hydroxylase activity. A characteristic of the biphenyl 2-hydroxylase is that, unlike most drug oxidation reactions (including biphenyl 4-hydroxylation) it is not totally dependent on NADPH being able to utilise NADH instead. Thus, on preincubation of hepatic microsomes with safrole in the presence of NADH instead of a NADPH-regenerating system, no enhancement of biphenyl 2-hydroxylation was observed although control values of this enzyme activity were unaffected by the change of cofactor. Presumably the explanation of this finding is that in the presence of NADH, safrole was not converted to an active metabolite. The length of preincubation time was also found to determine the degree of enhancement of the biphenyl 2-hydroxylase activity, optimal stimulation for safrole occurring after 30 min.

The quantitative significance of the figures quoted in Table 1 must be interpreted with caution for it is clear that the degree of enhancement observed is dependent not only on the rate of biological activation of the carcinogen but also on the concentration of carcinogen added. Thus, for 1 mM benz(a)pyrene maximal enhancement is observed within 5 min whereas for acetamidofluorene maximal enhancement is not achieved within 1 h.

Superficially, some correlation exists between the enhancement of biphenyl 2-hydroxylase activity and the degranulation of the endoplasmic reticulum but there are marked differences in other features of these two systems. For example, the carcinogen invoked biphenyl 2-hydroxylase enhancement is more rapidly mediated than degranulation; is considerably more susceptible to storage damage; and is not affected by EDTA (Table 1) which efficiently degranulates the rough endoplasmic reticulum¹¹. The rapidity and relative lability of the carcinogen-stimulated increase of biphenyl 2-hydroxylase activity suggests that the carcinogens are modifying the membranes of the endoplasmic reticulum, perhaps invoking a conformational change which might subsequently lead to membrane damage.

These results indicate that the study of the *in vitro* enhancement of biphenyl 2-hydroxylase in hepatic microsomes in the presence of a NADPH-regenerating system may prove a basis for a preliminary screening system which, in conjunction with other such tests, might be of considerable value in detecting potential carcinogens.

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Correlation of erythrocyte catechol-O-methyltransferase activity between siblings

CATECHOL-O-METHYLTRANSFERASE (COMT) is the enzyme that catalyses the conversion of noradrenaline, adrenaline, dopamine, and other catechol compounds to their O-methylated metabolites¹. COMT is widely distributed in many tissues including the red blood cell (RBC)^{1,2} and individual differences in the activity of this enzyme in human beings may be involved in the pathogenesis of various psychiatric and neurological diseases^{3,4}. Inhibitors of COMT have been used experimentally in the treatment of Parkinson's disease and other neurological diseases⁵. COMT activity is decreased in red blood cells obtained from depressed women³ and is increased in erythrocytes obtained from children with Down's syndrome⁶.

Although there has been interest in the determination of erythrocyte COMT activity in blood from patients suffering from neurological and psychiatric disease, little is known about factors that regulate RBC COMT activity in unselected human populations. Here we describe a study of erythrocyte COMT activity in blood obtained from a large, unselected population of young men and women, the results of which demonstrate a significant correlation of COMT activity between siblings and suggest that there may be at least two human populations with regard to levels of activity of this catecholamine degradative enzyme activity in the erythrocytes.

Blood samples were obtained from 373 unselected adolescents aged 16–18 yr as part of a survey of lipoprotein values in students attending the Rochester, Minnesota schools. All but one of the individuals studied were white. Blood was obtained at school in the morning after an overnight fast.

Blood samples were withdrawn by venipuncture into plastic syringes and were transferred immediately to 2 ml heparinised vacuum tubes. COMT activity was determined in every sample on the day that the blood was obtained by use of a radiochemical enzymatic assay that is a modification of the method described by McCaman⁷ (Fig. 1). 3,4-Dihydroxybenzoic acid was used as a substrate for COMT. One unit of enzyme activity represented the formation of 1 nmol of 4-hydroxy-3-methoxybenzoic acid (vanillic acid) per ml of packed red cells per hour of incubation. All results were evaluated by standard statistical methods.

Figure 1 shows the frequency distribution of erythrocyte COMT values in blood obtained from 373 unselected young men and women aged 16–18 yr. The mean COMT activity for the entire population was 11.3 ± 4.2 (mean $\pm$ s.d.); that of females was 10.9 ± 3.9 ; and the mean RBC COMT activity of the male subjects was 11.8 ± 4.5 . The distribution of COMT activity in this population seems to include at least two subgroups, one with a mean COMT activity of approximately 6–7 units and another with a higher mean enzyme activity of 12–14 units. This bimodality is present in the distribution of enzyme activities of the population as a whole and among the COMT activities determined in blood from the 189 female subjects and the 184 male subjects included in the study (Fig. 1). The same bimodality in the distribution of erythrocyte COMT values was found in a separate study of RBC COMT activity in 257 adolescents, 115 girls and 142 boys, age 13–15 yr in which COMT activity was determined in somewhat different assay conditions. In this younger population, as in the age 16–18 yr group, bimodality was present in the distribution of COMT activity of the entire population as well as in the separate distributions determined for male and female subjects.

A highly significant correlation ($r=0.485$) was found between erythrocyte COMT activity present in blood obtained from the 56 sibling-sibling pairs present in the population of young men and women aged 16–18 yr ($P<0.001$). The scatter diagram in Fig. 2 illustrates the sibling-sibling correlation of erythrocyte COMT values in this population. There was no difference in the degree of correlation between brother-brother, brother-sister and sister-sister pairings. When random pairs of single children with no siblings were generated by using tables of

random numbers, no significant correlation of erythrocyte COMT activity was found between members of non-sibling pairs. A highly significant correlation ($r=0.59$, $P<0.001$) was also present in blood obtained from the 37 sibling-sibling pairs included among the 257 adolescents aged 13–15 yr who were studied.

One of the difficulties in the determination of COMT activity in erythrocytes is the possibility that the results obtained might be due partially to the presence of endogenous inhibitors or activators of the enzyme. Both erythrocytes and plasma

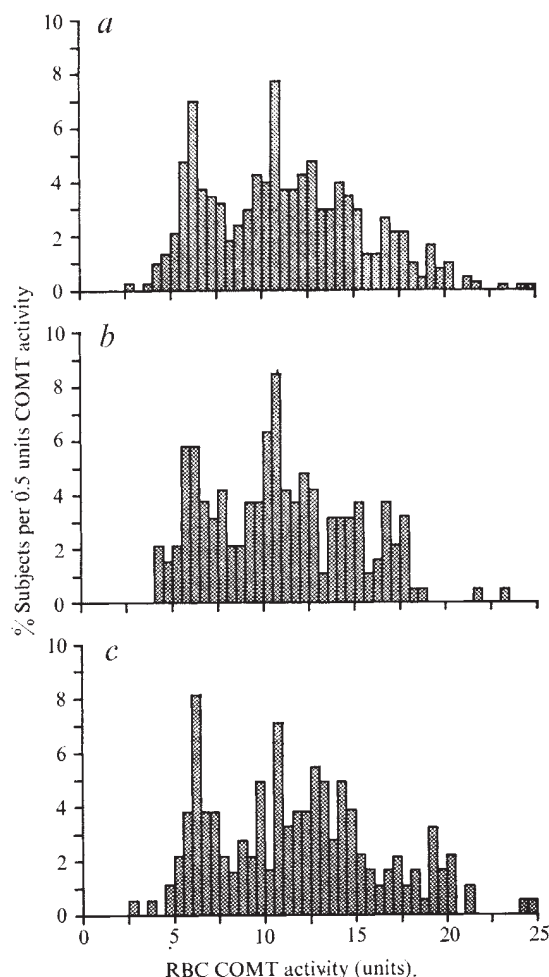


Fig. 1 Percentage frequency distribution of RBC COMT activity. The percentage of subjects with RBC COMT activity in successive 0.5 unit increments is shown for unselected populations that include: *a*, 373 males and females age 16–18 yr; *b*, 189 females and, *c*, 184 males considered separately. COMT activity was determined by a modification of the method of McCaman⁷. 200 μ l of heparinised whole blood were added to 800 μ l of ice-cold glass-distilled water containing 100 μ l of a suspension of sodium Chelex-100. The lysed blood samples were mixed with the resin by rotation at 12 r.p.m. for 1 h at 4°C. After centrifugation at 700g for 10 min the supernatant was removed for the determination of COMT activity. 200 μ l of the supernatant were placed in 15 ml stoppered conical centrifuge tubes to which 100 μ l of a mixture of the following reagents (final concentrations indicated) were added: Tris-HCl buffer, pH 7.5, 0.08 M; $MgCl_2$, 10^{-3} M; dithiothreitol, 4.2×10^{-3} M; ^{14}C -methyl-S-adenosyl-1-methionine (specific activity 58 μ Ci μ mol⁻¹), 2.8×10^{-6} M; non-radioactive S-adenosyl-1-methionine HCl, 20.2×10^{-6} M; 3,4-dihydroxybenzoic acid, 10^{-3} M. The reaction mixture was incubated for 45 min at 37°C, and the reaction was stopped with 100 μ l of 1 N HCl. The product was extracted into 5 ml of toluene. After centrifugation, 3.5 ml of the organic phase were removed and added to liquid scintillation counting vials that contained 10 ml of toluene fluor. The radioactivity of the samples was determined by liquid scintillation counting. Blank samples included all reagents except 3,4-dihydroxybenzoic acid. Haematocrits were determined on all blood samples, and results were expressed as nmol of vanillic acid formed per ml of packed red blood cells per hour. (Details of the method will be published elsewhere¹⁵.)

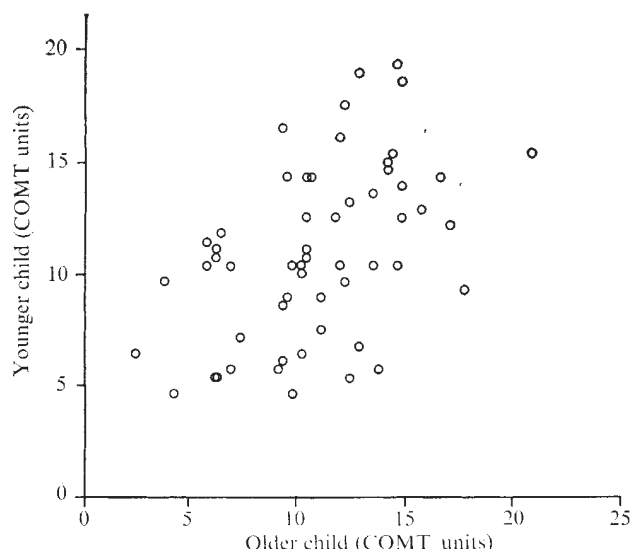


Fig. 2 Sibling-sibling correlation of RBC COMT activity. RBC COMT activity determined in blood from the younger sibling in a pair is plotted against the activity of RBC COMT in blood of the older sibling. $r=0.4846$; $P<0.001$.

contain magnesium, an ion that is essential for COMT activity¹. Variations in the concentrations of proteins that bind either endogenous or exogenously added magnesium might also influence enzyme activity. Blood contains S-adenosyl-1-methionine⁴, a cosubstrate for the COMT reaction¹. As the enzyme activity in this study was determined by the use of radioactively labelled methyl-¹⁴C-S-adenosyl-1-methionine, variable quantities of endogenous S-adenosyl-1-methionine in the blood might influence the apparent activity of the enzyme measured in a given individual. Finally, an enzymatic activity in blood that is capable of demethylating the product of the COMT reaction has been described⁸. Individual variations in this enzyme activity might also introduce artefacts into the determination of RBC COMT activity.

To help ensure that the individual differences in erythrocyte COMT activity reported here represent differences in the activity of the enzyme and not variations in the concentration of divalent ions, endogenous S-adenosyl-1-methionine, or other unknown inhibitors or activators of the enzyme, partially purified rat liver COMT was added to 201 consecutive blood samples in which RBC COMT activity was determined. Rat liver enzyme was purified by a modification of the method of Axelrod and Tomchick¹ as described by Coyle and Henry⁹ with the addition of a final step in which the enzyme was subjected to gel filtration on a G-200 column. The specific activity of this preparation was 551 nmol product per mg protein per hour incubation. The average recovery of rat liver COMT added to blood samples was $99 \pm 1.3\%$ (mean $\pm$ s.e.m.), and there was no difference in the recovery of exogenously added enzyme from samples with low or high endogenous COMT activity. In addition, when lysed blood samples of high COMT activity were mixed with lysates of low activity, the resultant enzyme activity was the expected arithmetic mean. The distribution of COMT values described here cannot be explained by an increased inhibition or decreased activation of samples with low activity as compared with lysates of high COMT activity, nor is the reverse true of samples with high COMT activity.

The factors that influence the activity of red blood cell COMT in erythrocytes from unselected human subjects are not well understood. The results of our study show that a significant sibling-sibling correlation occurs in RBC COMT activity. Thus, familial factors seem to be important in the regulation of levels of this enzyme in the human erythrocyte. The relative

contributions of heredity and shared environment to these familial factors, and the possible mechanism of inheritance (single gene or polygenic) will have to be determined by family and twin studies. The results of this study also suggest the existence of at least two subgroups of subjects in an unselected population, one with low and one with higher RBC COMT activity.

These results raise the possibility of significant familial differences in enzymatic O-methylation of catecholamines in man. It has been reported previously that the activity of another catecholamine degradative enzyme, monoamine oxidase in human platelets, is subject to genetic control^{10,11}. The serum activity of the catecholamine biosynthetic enzyme dopamine- β -hydroxylase (DBH), the enzyme that converts 3,4-dihydroxyphenylethylamine (dopamine) to noradrenaline, is affected by genetic factors. A striking sibling-sibling correlation exists for serum DBH activity¹²; this correlation is greater in blood samples obtained from monozygotic than in those obtained from dizygotic twin pairs¹³; and the results of recent studies are compatible with the existence of an autosomal recessive allele for very low serum DBH activity in man with a gene frequency of approximately 20% in the population studied¹⁴. The data that have been presented here with regard to a sibling-sibling correlation of COMT activity may represent an additional example of the importance of familial factors in the regulation of the activities of catecholamine biosynthetic and degradative enzymes in man. Although RBC COMT is biochemically similar to COMT in other tissues⁵, the identity of erythrocyte COMT with COMT in other human tissues has not been established. Further studies will be necessary to determine whether the findings reported here bear a relationship to a possible role of catechol-O-methyltransferase in human disease states.

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A reappraisal of human β MSH

A PEPTIDE containing 22 amino acids has been isolated from human pituitaries and, because of its structural resemblance to the octapeptide hormones previously isolated from pig, ox and sheep pituitaries and its similar pigment-dispersing biological activity in lower vertebrates, has been called β -melanocyte stimulating hormone (β MSH)¹. The discovery and structural analysis of two related sheep pituitary peptides^{2,3} β - and γ -lipotrophin (β LPH and γ LPH)¹ which showed that γ LPH comprised the first 1–58 amino acids of β LPH and, β MSH of sheep origin, the 41–58 sequence of both these molecules (Table 1), led Chretien and Li to suggest that β MSH was formed by enzymatic cleavage of β LPH, γ LPH representing the intermediate compound.

It has been shown that the human pituitary contains peptides identical to β lipotrophin and γ lipotrophin^{4,5}, and it is evident that 'human β MSH', although it differs in length from the other mammalian β MSHs, is also derived by cleavages of these molecules. The very close correspondence of the human β MSH sequence to the 37–58 region of sheep β LPH (and what is known of the structure of human β LPH (ref. 4)) can be seen in Table 1.

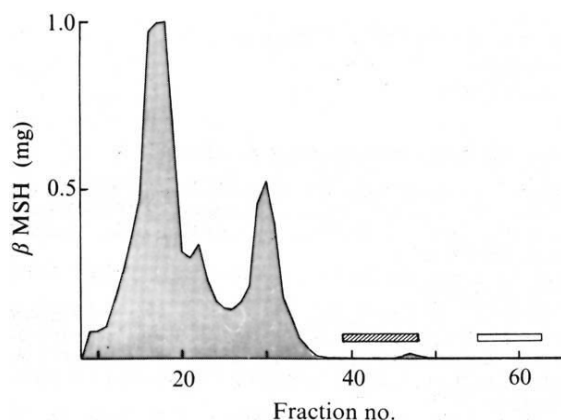


Fig. 1 Fractionation of a human pituitary gland extract on a BioGel P6 column eluted with 1 M acetic acid (column dimensions 90 cm $\times$ 1.5 cm). The shaded area represents β MSH immunoreactive material. The cross-hatched block indicates the elution position of ACTH and the open block that of synthetic human β MSH.

In comparing the β MSHs of the different species, it was apparent to us that as well as its larger size, human β MSH shows other peculiarities not shown by the other mammalian β MSHs. It is more difficult to extract⁶ and is localised in the pars distalis rather than the pars intermedia⁷—a lobe which is rudimentary in man. These anomalies have persuaded us that the nature and role of the human β MSH molecule needs a more critical reappraisal. Here we describe our preliminary findings, which seem to throw considerable doubt on the actual existence

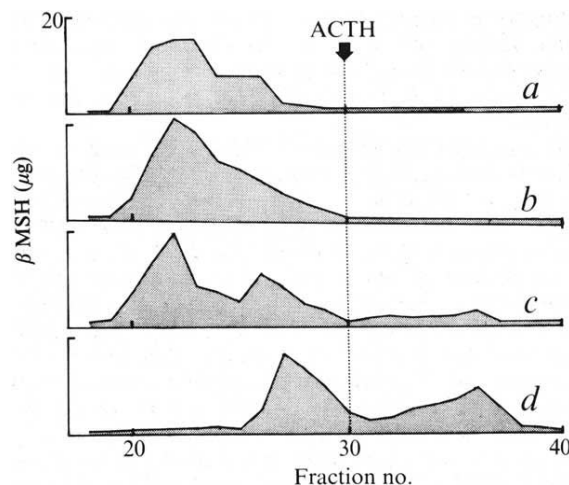


Fig. 2 Samples were removed at timed intervals from a 1 M acetic acid extract of human pituitaries, boiled to prevent further enzyme activity and chromatographed on a BioGel P6 column (90 cm $\times$ 1.5 cm). The distribution of β MSH-like immunoreactive material in each fraction is indicated by the shaded peaks. The vertical dotted line represents the approximate elution position of ACTH, and serves as a marker to show that prolonged extraction in 1 M acetic acid can lead to the formation of β MSH-like material of a smaller molecular size than ACTH. a, Zero time; b, 0.5 h; c, 2 h; d, 16 h.

of human β MSH, and indicate that it may be an artefact of the β LPH molecule formed during certain extraction procedures.

A radioimmunoassay for human β MSH, employing a synthetic human β MSH preparation (CIBA/Giegy) for standardisation and iodination, was developed, and cross-reaction with ACTH and α MSH was shown to be less than 0.1%. Human β and γ LPH were shown to be equipotent on a molar basis in this assay, and gave curves parallel to the standard.

Human pituitaries (frozen or acetone dried) were extracted in several different ways, assayed, and the extracts applied to BioGel P6 columns and eluted with acetic acid⁸. The pituitaries contained substantial amounts of β MSH immunoreactive material at concentrations corresponding to those reported by Abe and his colleagues⁸. After gel filtration, however, none of the β MSH immunoreactive material behaved as expected for human β MSH, but appeared as high molecular weight material eluting in fractions before ACTH, which has 39 amino acid residues (Fig. 1). Two peaks were identified and further purified by ion-exchange chromatography⁵. The largest molecular weight peak, which predominated in all extracts studied, had an amino acid composition closely resembling β LPH, and the other peptide resembled the NH_2 -terminal 58 amino acid sequence of human β LPH (γ LPH).

Further studies suggested that β MSH was only derived by enzymatic cleavage of these molecules during certain extraction procedures, most commonly those involving the use of dilute

Table 1 Amino acid sequences of human and ovine β LPH, γ LPH and β MSH.

	1	37	60	90
β LPH (NH_2)	ALA-GLU-LYS-LYS-ASP-SER-GLY-PRO-TYR-LYS	MET-GLU-HIS-PHE-ARG-TRP-GLY-SER-PRO-PRO-LYS-ASP-LYS-ARG		(COOH)
	not determined	LYS	GLU	ARG
β MSH	H-ALA-GLU-LYS-LYS-ASP-GLU-GLY-PRO-TYR-ARG	MET-GLU-HIS-PHE-ARG-TRP-GLY-SER-PRO-PRO-LYS-ASP-OH		Human
		(1)		
	H-ASP-SER	LYS	(18)	Sheep
γ LPH (NH_2)	ALA-GLU-LYS-LYS-ASP-SER-GLY-PRO-TYR-LYS	MET-GLU-HIS-PHE-ARG-TRP-GLY-SER-PRO-PRO-LYS-ASP-OH		Sheep
			58	

The sequence of seven amino acids enclosed by the vertical dotted lines is identical to that of α_{1-30} ACTH. This is the 'core' responsible for the melanocyte-stimulating activity of β MSH, γ LPH, ACTH and α MSH.

acid. For example, the β LPH in human pituitaries was readily degradable in extracts prepared using 6% acetic acid, unless enzyme activity in the tissue was destroyed by heating or lowering the pH (Fig. 2). A proportion of the degraded fragments resembled human β MSH in their physicochemical properties.

Our interpretation of how β MSH was found in human pituitaries is exceptionally well supported in the literature. It is evident, for example, that the original isolation of human β MSH was made from a side-fraction of a growth hormone extraction procedure¹, and it seems quite probable, as a result of the employment of mild extraction conditions to obtain the growth hormone, that degradation of β LPH and γ LPH took place during this or subsequent procedures to form β MSH. Lerner and his colleagues⁶ have reported several times in the past decade how, if direct extraction of pituitaries is carried out in strong acid conditions (conditions which do not favour proteolysis), human β MSH cannot be isolated.

We have recently been extending our studies to immuno-reactive β MSH in human plasmas, samples of which have now been taken from patients with Nelson's syndrome and a normal subject following pyrogen administration. In all cases, the concentrations of β MSH immunoreactive material were very high, but on gel filtration none of this material eluted in the expected position of human β MSH (Fig. 3). The bulk of immunoactivity resembled β LPH and/or γ LPH in its elution properties, but some was always found in the void volume (a molecular weight of greater than 20,000 was indicated). This latter material may be either an even larger precursor molecule, (such as 'Big ACTH'), or it may be β LPH non-covalently linked to a plasma protein.

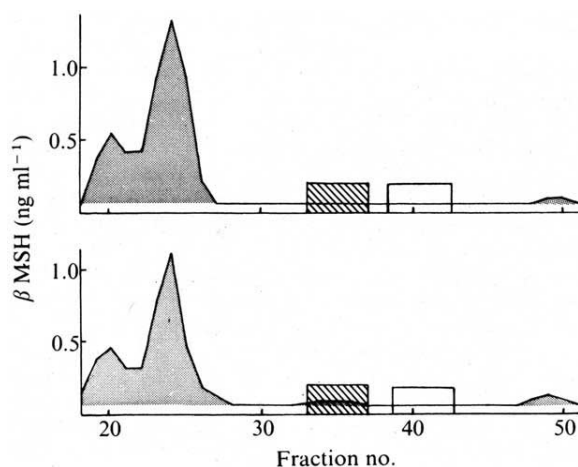


Fig. 3 Fractionation of plasma from two patients with Nelson's syndrome on a BioGel P6/P10 column eluted with frog-Ringer/albumin buffer. (Column dimensions: P6, 100 cm $\times$ 1 cm, P10, 33 cm $\times$ 1 cm.) The shaded peaks represent β MSH immunoreactive material. The cross-hatched block indicates the elution position of ACTH and the open block that of synthetic human β MSH. Most of the β MSH immunoreactivity eluted in a peak corresponding in molecular size to β LPH.

The stability of β MSH immunoactivity in fresh plasma, thawed plasma or whole blood bears a much closer similarity to the behaviour of β LPH and γ LPH than to synthetic human β MSH, when they are exogenously added to the fluids. The larger peptides are considerably more stable than the β MSH.

In conclusion, our findings suggest that it is likely that radioimmunoassays for human β MSH cannot actually be measuring this hormone, as it does not normally exist. It seems instead that human β MSH radioimmunoassays will be measuring other peptides, the most important of which in pituitary tissue is β lipotrophin, and in plasma, either β LPH or γ LPH. This

conclusion readily explains why human β MSH differs so much from the other mammalian β MSHs. It is especially evident that the well-documented association between β MSH and ACTH in man¹¹ (the two peptides appear to be synthesised and stored in the same pituitary cell and released together in the same physiological or pathological situations) is really an association between β LPH and ACTH.

A peptide resembling a true β MSH has been found in extracts of several tumours associated with the ectopic ACTH syndrome^{12,13}. On gel filtration, this peptide elutes in the same position as a bovine β MSH standard, but not in the position of the synthetic human β MSH standard. This finding is not unexpected, as it ties in very well with our findings on the other melanophore stimulating hormone, α -MSH, and the 'corticotrophin-like intermediate lobe peptide' (CLIP), both of which are derived by cleavage of ACTH in pituitary pars intermedia cells¹⁴, and are also found in tumours associated with the ectopic ACTH syndrome in man¹⁵.

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Possible pharmacological and theoretical implications of X-ray structure of the tricyclic antidepressant imipramine

IMIPRAMINE was first shown¹ in 1958 to be effective in the treatment of endogenous depression. It is one of the most widely prescribed members of the tricyclic antidepressant group of compounds which have been chosen for the treatment of the average depressed patient². It is perhaps surpris-

ing that not a single member of this important group of psychotropic drugs has been investigated by X-ray analysis. There is now considerable evidence that a functional abnormality of one or more of the biogenic amine systems of the brain is involved in the aetiology of the depressive or affective disorders³. Amines which have been investigated most thoroughly in this respect are noradrenaline⁴ and 5-hydroxytryptamine⁵ (5-HT). The synaptic action of these putative

neurotransmitters is thought to be terminated largely by an active uptake back into the presynaptic nerve ending, and the rat brain is known to possess separate neuronal uptake systems for noradrenaline⁶ and 5-HT⁷. The most striking neurochemical effect of tricyclic antidepressants is their potency in inhibiting these transport processes⁸, and indeed it has been suggested that this effect may be responsible for their clinical ameliorative action⁴. Structure-activity relationships for various imipramine analogues as inhibitors of noradrenaline⁹ and 5-HT^{10,11} uptake have been reported and recently a possible molecular mechanism for this inhibitory effect has been proposed¹². In an attempt, therefore, to gain some insight into the conformational requirements for a compound to be a potent inhibitor of noradrenaline and 5-HT uptake, we are investigating the X-ray structures of a number of tricyclic antidepressants¹³; that of imipramine is reported here.

Well-formed crystals of imipramine hydrochloride, ranging from 0.1–1.5 mm in size in the largest dimension, were grown from a chloroform–xylene mixture. The space-group was uniquely determined as $P2_1/c$ with eight molecules in the monoclinic cell of dimensions $a=11.303$ Å, $b=29.277$ Å, $c=14.282$ Å, $\beta=130.91^\circ$, thus requiring two distinct molecules in the crystallographic asymmetric unit. This gives rise to the interesting possibility of two conformations in the solid state structure. Diffractometer data were collected and normalised structure factors (E_s) derived. The structure was solved using reflections with $E \geq 1.2$ by a centrosymmetric multi-solution direct methods program (G. M. Sheldrick, private communication). At present, the conventional R factor is 0.057; complete details of the fully refined structure will be published elsewhere. Figure 1 illustrates the close similarity of the geometry of the tricyclic ring system for the two molecules of the asymmetric unit. The dihedral angle between the planes of the benzene rings is 130° and 123° for molecules A and B, respectively, while the angles between each benzene ring and the plane N—C(methylene)—C(methylene) of the seven-membered ring are 18° and 116° (for A) and 2° and 125° (for B), the latter set of values reflecting the twist in the ring system. The ring nitrogen atom is pyramidal, lying 0.19 Å (for A) and 0.29 Å (for B) from the plane formed by the three carbon atoms to which it is bonded.

The dimethylaminopropyl chains show a definite variation in conformation. The values approximate to a staggered conformation with respect to the N—C(benzene) bonds. From τ_1 onward, A is almost fully extended, that is, all torsion angles are close to $\pm 180^\circ$, while B folds back to τ_3 . In each case the amino nitrogen is hydrogen bonded to a Cl ion 3 Å distant. As the tricyclics are structurally dissimilar to noradrenaline and 5-HT, it is interesting to know what structural and conformational properties enable them to be such potent inhibitors of the neuronal transport of these two putative CNS transmitters. Kinetic studies indicate that they are competitive inhibitors of these uptake systems in the peripheral¹⁴ and central nervous systems⁹ and in blood platelets¹⁵, and are, therefore, probably binding to the same site as the endogenous substrates. The most obvious function for the terminal amino group of imipramine is presumably to block the binding site with which the primary amine function of noradrenaline or 5-HT normally interacts¹². It is possible that one of the benzene rings of the tricyclic nucleus blocks the binding site usually occupied by the aromatic ring of noradrenaline¹². Similarly, a larger portion of the tricyclic system may also effectively block the binding site of the indole nucleus of 5-HT. Previous studies^{16–18} have shown that the most likely conformation of phenylethylamine analogues and catecholamines at the uptake site is very similar to the fully extended *trans* form which has been shown to be the preferred conformation of noradrenaline in the solid state¹⁹, in solution²⁰ and *in vacuo* by theoretical calculations²¹. A comparison of the distances

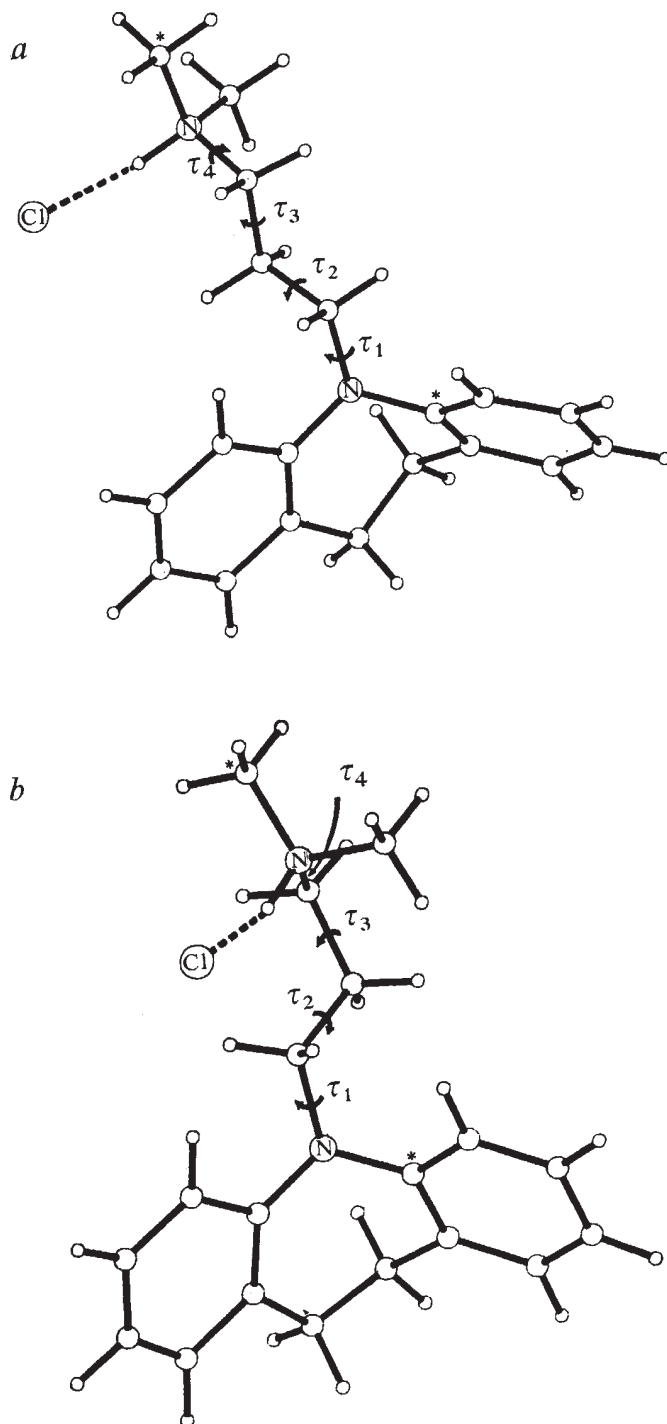


Fig. 1 Molecular diagrams of imipramine hydrochloride as determined by X-ray analysis. *a*, Molecule A; *b*, molecule B. An asterisk marks the atoms used in torsion angle calculations where an ambiguity in definition occurs. Torsion angle values for A and B are as follows (A is quoted first): $\tau_1=137^\circ$, 59° ; $\tau_2=180^\circ$, 161° ; $\tau_3=174^\circ$, 61° ; $\tau_4=169^\circ$, -172° .

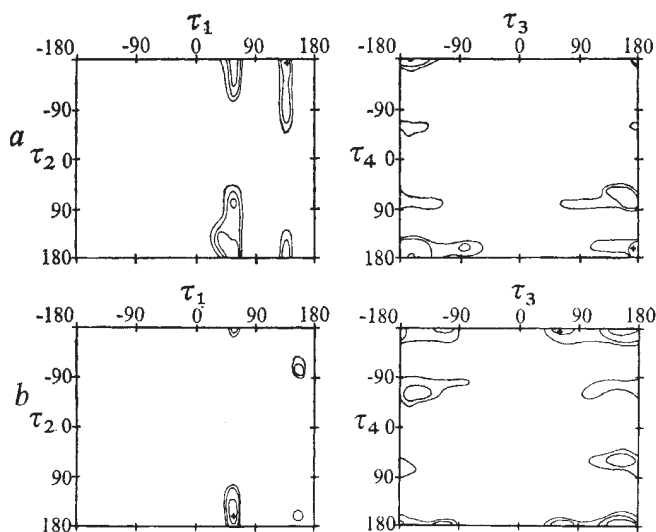


Fig. 2 Potential energy maps for imipramine. *a*, Molecule A; *b*, molecule B. Energies were calculated by atom-atom summation of van der Waals interactions as programmed by Motherwell²³. Contours are at 1 kcalorie mol⁻¹ intervals and areas not contoured are regions of high energy corresponding to close interatomic approaches and atomic collisions. Heavy crosses indicate the conformations found in the crystal structure.

of the terminal nitrogen atom from the centres of the aromatic rings in both conformations of imipramine with that in noradrenaline¹⁹ shows that in the case of conformation B the above proposals¹² seem reasonable, although exact coincidence is not attained and may not in fact be necessary. The possibility of conformations other than those found in the crystal, existing in solution can not be excluded (as a recent NMR study of imipramine and its hydrochloride has shown²²) and a potential energy approach toward this problem has been applied.

Figure 2 shows potential energy maps obtained by varying, in each case, two torsional angles of the molecular side chain whilst the remaining two are fixed, in this particular case, at values observed in the X-ray analysis, to reduce the problem to one in two dimensions. This limitation is not a prerequisite, however, and the same approach can be used iteratively, without any previous assumption of τ values, to study the complex arrangement of more than two variables. It is apparent from Fig. 2 that other conformations of similar energy to that of the crystal form could exist, as indicated by a number of minima in the maps other than those corresponding to the crystal position. There seems to be no *a priori* method of distinguishing which conformation is most likely in the absence of packing constraints (which are important in the crystalline form) and further studies with more rigid tricyclic antidepressants, which are currently underway in our group, may throw light on this problem.

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Transcriptional control of α -foetoprotein synthesis in developing mouse liver

α -FOETOPROTEIN (α FP) is a dominant serum protein in many mammalian species during embryonic and early postnatal life. It is hardly detectable in the adult body but is often found in an elevated quantity in the serum of individuals with hepatocarcinomas and teratocarcinomas¹⁻⁵.

α FP is synthesised in the yolk sac and foetal liver but it is not known whether the decrease in α FP synthesis in adult liver is due to an alteration in mRNA synthesis or in mRNA translation. To answer this question we have extracted RNAs from polysomes of foetal and adult mouse liver and measured their abilities to direct the synthesis of α FP in cell-free systems. Under the conditions used, the translation of polysomal RNA depends on the presence of high-salt extracts of ribosomes (ribosomal wash) which are known to contain factors involved in the initiation of polypeptide synthesis. It has been possible to determine whether the reason for the reduction in α FP synthesis in adult liver resides in the mRNA fraction or in the ribosomal wash.

Total polysomes were prepared from the liver of adult ICR mice of 14 day foetal mice according to Falvey and Staehelin⁶. RNA was extracted from the polysomes using 1% sodium dodecyl sulphate (SDS) and a mixture of phenol and chloroform⁷. In some experiments, polysomal RNA was further fractionated by treating the total polysomes with 1% SDS, 5 mM EDTA and 20 mM NaCl followed by sucrose density-gradient centrifugation⁸. RNA sedimenting at 15-20S

Table 1 Synthesis of α FP with ribosomes and high speed supernatant from foetal and adult liver

Ribosome	High speed supernatant	Foetal polysomal RNA	α FP (c.p.m.)
Foetal	Foetal	+	387
Foetal	Adult	+	411
Adult	Adult	+	396
Foetal or adult	Foetal or adult	—	93

The components for protein synthesis were described in the legend to Fig. 1. The samples were incubated at 30° C for 60 min and centrifuged at 149,000g for 2 h. Immunoprecipitation was carried out using 500 μ l samples as described previously¹⁴ with the following modifications. The reaction was performed in the presence of 1% Triton X-100 and 1% sodium deoxycholate (DOC) to minimise non-specific precipitation¹⁶⁻¹⁸. The samples were then centrifuged through a 0.9 M sucrose cushion containing 1% Triton X-100, 1% DOC and 10 mM each of leucine, valine and phenylalanine. The pellets were dissolved in 1% sodium dodecyl sulphate-4 M urea-1% β -mercaptoethanol at 45° C for 30–60 min and the radioactivity was measured with 10 ml Aquasol (NEN Canada, Montreal, Quebec).

Figures are averages of three assays.

was recovered by precipitation with ethanol and used as 18S RNA. This RNA fraction was enriched with α FP mRNA (K. K., D. W. O., T. I., and T. T., unpublished) as well as albumin mRNA⁹⁻¹¹. Ribosomal wash was prepared according to Prichard *et al.*¹². The cell-free systems from the adult mouse liver (adult liver cell-free system) and 14 day foetal liver (foetal liver cell-free system) were prepared as described previously¹¹.

Figure 1 shows polyacrylamide gel electrophoretic analysis of proteins produced in the foetal liver cell-free system in the presence of foetal polysomal RNA and foetal ribosomal wash. Four main radioactive fractions were resolved, two of which were comigrating with carrier α FP and albumin, respectively. Additional evidence that they were in fact newly formed α FP and albumin was obtained by immunoprecipitation followed by gel electrophoresis^{13,14}. Control samples incubated without polysomal RNA or ribosomal wash showed little radioactivity throughout the gel.

The requirements for α FP synthesis in the cell-free system were studied by substituting foetal liver components with corresponding preparations from adult liver. In this experiment, α FP synthesis was assayed by measuring radioactivity in the precipitate formed with antiserum against mouse α FP. Specificity of the reaction was examined using various control samples and will be published elsewhere. Table 1 shows that substitutions of foetal ribosomes and high speed supernatant fraction with adult counterparts did not affect the synthesis

Table 2 Synthesis of α FP and albumin in the adult liver cell-free system

Exp.	RNA	Ribosomal wash	α FP (c.p.m.)	Albumin (c.p.m.)	α FP/Albumin
I	Foetal	Foetal	335	130	2.6
	Foetal	Adult	201	76	2.6
	Adult	Foetal	54	167	0.3
	Adult	Adult	25	120	0.2
II	Foetal	Foetal	288	126	2.3
	Foetal	Adult	248	83	3.0
	Adult	Foetal	79	147	0.5
	Adult	Adult	66	105	0.6

Conditions for protein synthesis were the same as described in the legend to Fig. 1, except that 'run-off' 80S ribosomes and high speed supernatant were prepared from adult mouse liver. The adult ribosomal wash was used at 1.5 mg protein ml⁻¹. In I and II, the '18S' RNA from foetal and adult polysomal RNA was used at 3.5 A_{260} ml⁻¹. The samples were incubated at 30° C for 60 min and centrifuged at 149,000g for 2 h. The supernatant was removed and divided into two aliquots. One (500 μ l) was analysed for α FP as described in the legend to Table 1. The other was analysed for albumin in a similar manner using rabbit antiserum against albumin. Appropriate background counts, obtained in the absence of RNA (95–190 c.p.m.), were subtracted.

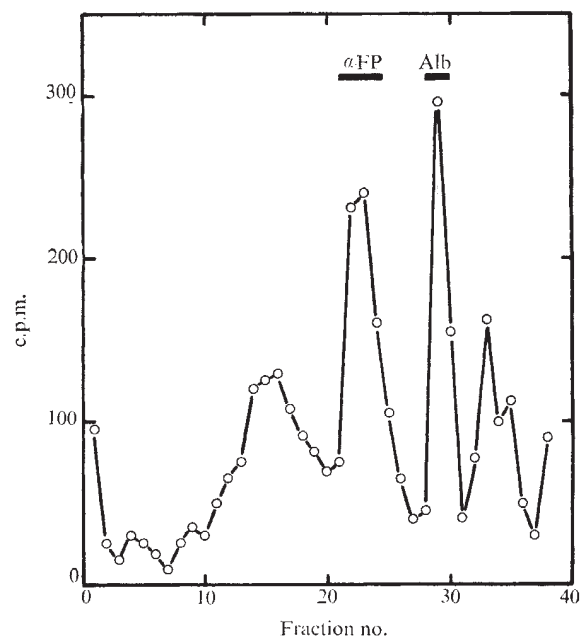


Fig. 1 Polyacrylamide gel electrophoretic analysis of proteins produced in the presence of foetal polysomal RNA in the foetal liver cell-free system. The reaction mixture (3 ml) contained the following (per ml): 2 A_{260} foetal 'run-off' 80S ribosomes, 6 mg foetal high-speed supernatant protein, 1 μ mol ATP, 0.3 μ mol GTP, 10 μ mol phosphocreatine, 50 μ g mouse liver tRNA, 35 μ Ci ³H-leucine (1 Ci mmol⁻¹), 10 μ Ci ³H-valine (2 Ci mmol⁻¹), 10 μ Ci ³H-phenylalanine (6.15 Ci mmol⁻¹), 25 nmol each of 17 other amino acids, 20 mM Tris-HCl, pH 7.8, 3.5 mM MgCl₂, 70 mM KCl, 6 mM β -mercaptoethanol, 0.5 mM dithioerythritol, 750 μ g foetal ribosomal wash protein and 3.5 A_{260} foetal polysomal RNA. The sample was incubated at 30° C for 60 min and centrifuged at 149,000g for 2 h, the supernatant adjusted to pH 4.8 and the precipitate removed. (NH₄)₂SO₄ was added to the supernatant to 40% saturation at 0° C and the precipitate was dissolved in 20 mM Tris-HCl (pH 7.8)–150 mM NaCl. This resulted in about fourfold purification of α FP and albumin¹¹. Electrophoresis was performed with neuraminidase-treated α FP and albumin as carriers^{14,19} and radioactivity was measured as described previously^{13,14}. The bars indicate positions of carrier α FP and albumin (Alb).

of α FP. This suggested that either mRNA or ribosomal wash was responsible for the regulation of α FP synthesis.

The synthesis of α FP in the presence of foetal or adult polysomal RNA in combination with foetal or adult ribosomal wash, was therefore examined, using an assay of the synthesis of albumin as an internal control. The results showed that when foetal polysomal RNA was used as a template, α FP synthesis exceeded albumin synthesis two- to threefold with either foetal or adult ribosomal wash (Table 2). When adult polysomal RNA was used as a template, however, the synthesis of α FP decreased drastically with either foetal or adult ribosomal wash. Albumin synthesis was relatively unchanged,

Table 3 Synthesis of α FP and albumin in the mouse sarcoma cell-free system

RNA	Ribosomal wash	α FP (c.p.m.)	Albumin (c.p.m.)	α FP/Albumin
Foetal	Foetal	308	133	2.3
Foetal	Adult	365	145	2.5
Adult	Foetal	263	322	0.8
Adult	Adult	99	164	0.6

Conditions for protein synthesis were the same as that described in the legend to Fig. 1, except that 200 μ l S-30 of mouse sarcoma 180 replaced 'run-off' 80S ribosomes and high speed supernatant. Both foetal and adult RNA preparations used were total polysomal RNA (3.5 A_{260} ml⁻¹). The samples were incubated at 30° C for 2 h and 330 μ l post-ribosomal supernatant was used for the assay of α FP or albumin as described in the legend to Table 1. Appropriate background counts, obtained in the absence of RNA (106–225 c.p.m.), were subtracted.

Table 4 Synthesis of α FP and albumin in the presence of nuclear and cytoplasmic RNA from adult mouse liver

RNA	α FP (c.p.m.)	Albumin (c.p.m.)	α FP/Albumin
Nuclear	7	21	0.3
Cytoplasmic	18	184	0.1
Foetal polysomal	217	57	3.8

Assay conditions were the same as described in the legend to Table 3. RNA was used $3 A_{260} \text{ ml}^{-1}$. Appropriate background counts, obtained in the absence of RNA (105 c.p.m. for α FP, 156 c.p.m. for albumin), were subtracted.

and as a result, the ratio of α FP synthesis to albumin synthesis decreased to 0.2–0.6. This indicated that α FP mRNA was deficient in the adult polysomal RNA although the factors necessary for its translation were present in adult liver. The counts reported here were relatively low but a series of experiments showed that the results were highly reproducible as shown by two examples in Table 2. Higher counts of α FP have since been obtained by including ^3H -glutamic acid in the reaction mixture. This is presumably due to a high content of glutamic acid in α FP (E. F. Zimmerman, unpublished). These results confirm the conclusion stated above.

A similar experiment was conducted in a heterologous cell-free system using an S-30 fraction prepared from mouse sarcoma 180¹⁵. Translation of polysomal RNA in this system was also dependent upon the addition of ribosomal wash (K. K., D. W. O., T. I., and T. T., unpublished). Table 3 shows that the synthesis of α FP depended on the source of mRNA but not of ribosomal wash, in agreement with the results obtained with the liver cell-free system.

Although the above experiments indicated the deficiency of active α FP mRNA in the adult liver polysomes the possibility existed that it might be present in the nucleus or in the cytoplasm in a free form. This was tested by fractionating the adult liver into nuclei and cytoplasm, extracting RNA from each fraction and measuring its ability to synthesise α FP and albumin. Table 4 shows that the synthesis of α FP by the nuclear or cytoplasmic RNA was small, suggesting that the level of α FP mRNA in adult liver is in fact greatly reduced.

Another possibility, that α FP mRNA may have been degraded during extraction from adult liver, can be ruled out as albumin mRNA was extracted in an active form from the same source.

We conclude that the synthesis of α FP in the developing mouse liver is regulated at the level of mRNA. Several steps are involved in the production of mRNA and its appearance in the cytoplasm, for example, transcription, transportation, maturation and the addition of poly(A). Further study is necessary to determine which one of these reactions is responsible for the reduced synthesis of α FP mRNA in adult liver. It is conceivable that such control is relaxed in hepatomas and teratomas, leading to an elevated level of α FP in the serum. Elucidation of the regulatory mechanism involved may thus throw light on the process of malignant transformation as well as normal development.

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In vitro neoplastic transformation of epithelial cells of rat urinary bladder by nitrosamines

ORAL administration of N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN) selectively induces tumours in the urinary bladder of rats and mice^{1–3}. This organ specificity can be explained to some extent by the metabolic fate of the compound and the carcinogenic effect of its major urinary metabolite on the bladder; Okada and Suzuki⁴ found that BBN given to a rat was soon excreted as its carboxy derivative, N-butyl-N-(3-carboxypropyl)nitrosamine (BCPN). Subsequent tests for carcinogenicity of BCPN showed that it too caused bladder tumour in rats^{5,6}. Furthermore, BBN can be converted to BCPN when incubated with bladder mucosa or liver tissue⁷. These findings suggest that BCPN is responsible for carcinogenesis of the bladder by BBN, or that both BCPN and BBN are directly carcinogenic to bladder epithelium. We have therefore studied the *in vitro* effect of both BCPN and BBN on

Table 1 Culture of epithelial cells of urinary bladder in various media

Experiment series	Age of donors (weeks)	No. of flasks containing cell strains/No. of tested flasks with medium* of					
		A	B	C	D	E	F
0†	12, 15	0/6	0/4	0/4	0/4	0/4	0/4
1	12	0/1	0/1	0/1	2/2	0/1	2/2
3‡	8	0/1	0/1	0/1	2/2 (0/1)	0/1	1/1 (0/1)
6	9	0/1	0/1	0/1	1/2		
8‡	39	0/1	1/2	0/1	3/3 (1/1)	0/1	2/2 (1/1)
Total		0/10	1/5	0/8	8/9	0/7	5/5

A, Plain medium; B, plain medium plus urea; C, plain medium plus BCPN; D, plain medium plus urea and BCPN; E, plain medium plus BBN; F, plain medium plus urea and BBN.

*Concentration of BCPN or BBN was 0.03%.

†Accumulated results of preliminary experiments.

‡Data in parenthesis shown in series 3 and 8 indicate the results of culture in 0.015 and 0.06% nitrosamine-containing medium, respectively.

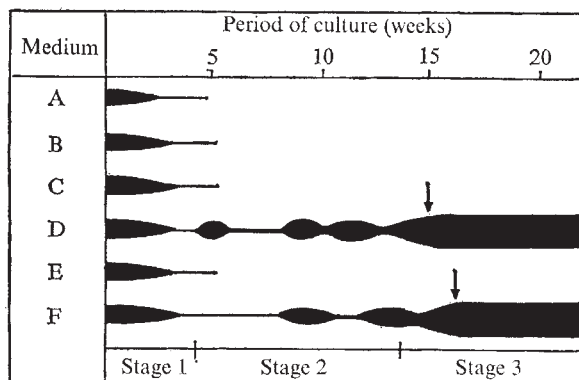


Fig. 1 Growth patterns of epithelial cells of the bladder in various media. Rats were killed by bleeding from the aorta. After terminal ligation of the bladder, about 1 ml of 0.25% trypsin solution containing 0.05% disodium salt of ethylenediaminetetraacetic acid was injected into the bladder lumen. The inflated bladder was separated from the animal and incubated in a humidified CO_2 incubator for 45 min at 37°C , and its contents were collected by cutting one end. The bladder was turned inside out and the mucosa was squeezed gently with forceps and washed with 10 ml of Eagle's minimal essential medium (MEM). A combined cell suspension of the contents and the washings of five bladders was transferred to centrifuge tubes, and placed in an ice bath for 3 min to remove sedimented debris and nematode worms. The cells were collected by centrifugation, washed once with MEM and suspended in MEM supplemented with 10% foetal calf serum and $60\text{ }\mu\text{g}$ kanamycin ml^{-1} (plain culture medium). Cell suspension containing 300×10^3 cells in 5 ml was inoculated into a square type tissue culture flask (MA 30) which was sealed with a rubber stopper. After culture for 3–4 d at 36°C the medium was replaced by medium A, B, C, D, E or F (described in the legend to Table 1). Nitrosamines were dissolved in physiological saline making 0.3% stock solutions, and urea was dissolved in MEM making 0.5% solution just before preparation of culture media. Concentrations of nitrosamine and urea in culture media were adjusted to 0.03 and 0.05%, respectively. Each medium was replaced every 7–10 d with the medium of the same constitution as the first. The black area in the figure indicates the apparent growth patterns of epithelial cells in experimental series 3 (see Table 1). The arrow indicates the time of passage.

epithelial cells of rat bladder. In preliminary experiments we had failed to induce growth of these cells, but we found that addition of urea to medium containing a nitrosamine induced growth. We have now established epithelial cell strains from normal cell by culturing them in the presence of BCPN plus urea or BBN plus urea and found that they grow as cancers in syngeneic animals.

Cells were obtained by trypsinisation from bladder mucosa of adult male rats of the inbred ACI/N strain (usually five of

the same age for each series of experiments). Cells were cultured in Eagle's minimum essential medium supplemented with 10% foetal calf serum and $60\text{ }\mu\text{g}$ kanamycin ml^{-1} (referred to here as plain culture medium) for 3–4 d until they had fixed and spread on the surface of a tissue culture flask. Then the cells were cultured in the presence of urea or BCPN or BBN or a nitrosamine plus urea without removing these compounds from the medium until the cell strain was established.

In plain culture medium epithelial cells became polynuclear

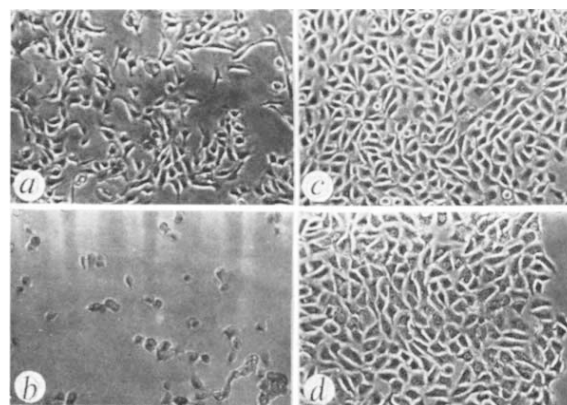


Fig. 2 Culture of cells in medium containing nitrosamine and urea. *a*, Initial cell growth in medium containing BCPN and urea at 5 weeks; *b*, degeneration of the cells at 6 weeks; *c*, cell strains established by BCPN and urea (BES3P) and *d* by BBN and urea (BES3B). (Phase contrast, $\times 100$.)

and died after 4–5 weeks (Fig. 1). In medium containing urea alone or a nitrosamine alone the fate of the cells was similar. In medium containing urea and BCPN or urea and BBN some cells survived for 4–8 weeks, although many died during this period (stage 1). The surviving cells started to divide after this latent period (Fig. 2*a*) and formed one or two colonies per flask. When a focal monolayer formed, many cells died in 1–2 weeks (Fig. 2*b*) (this is referred to here as contact death), but a few cells survived and divided again. This process was repeated two or three times (stage 2), and finally cells grew without contact death to form a confluent monolayer (stage 3). Usually it took 13–15 weeks for cells to reach the final stage. Confluent cells were trypsinised and passed to other flasks, thus establishing cell strains amenable to serial passage. Attempts to passage stage 2 cells, by trypsinisation or by scraping with a rubber policeman, resulted in rapid degeneration of the cells in a new flask. If medium containing both

Table 2 Backtransplantation of cells of established strains into syngeneic rats

Cell strain and passage no.	Period of culture (weeks)	X-irradiation to recipient	No. of cells injected ($\times 10^6$)	Injection site	No. of rats bearing tumour	Days until tumour examination
BES3B-6	24	—	0.1	i.p.	2/2	183, 183*
BES3B-7	25	—	1	i.b.	2/2	100(2)
BES3B-8	31	+	1	i.p.	1/2	64
BES3B-8	31	+	1	s.c.	2/2	64, 86
BES3P-4	25	—	2	i.b.	2/2	147, 212
BES3P-8	42	—	2	s.c.	3/3	128(3)
BES3P-8	42	+	2	s.c.	1/1	128
BES8B-6	31	+	5	i.p.	2/2	122, 128*
BES8B-6	31	+	5	s.c.	1/2	125
BES8P-5	31	+	5	i.p.	2/2	125, 128
BES8P-5	31	+	5	s.c.	0/2	
BES8U-2	27	—	2	s.c.	4/4	100(2), 128(2)

Cells, which had been kept by serial passages and by continuous culture in the medium containing nitrosamine and urea or urea alone, were cultured in several large flasks containing plain culture medium until they formed a confluent monolayer. Cells obtained by trypsinisation were suspended in Eagle's medium and counted. A known number of cells was injected into 8–9-week-old male ACI/N rats, some of them already irradiated with 300 r. The recipients were killed when tumours reached appropriate sizes for examination. Animals which did not develop tumours were observed until 200 d after transplantation. Cell strains are named as follows: BE means they derived from bladder epithelial cells; S number denotes the experimental series number, and the last character shows the drug added in the medium, that P, BCPN plus urea; B, BBN plus urea; U, urea alone. i.p., intraperitoneal; s.c., subcutaneous; i.b., into bladder wall.

*Rat died due to tumour on that day.

urea and nitrosamine was changed to plain culture medium at this stage, all cells died in several weeks without further growth, whereas cells in stage 3 or cells of the established strains could grow even in plain culture medium. Large fibroblast-like cells, which were always present with epithelial cells in the early stage of culture, grew to some extent, but always degenerated gradually and after 10 weeks few were detectable. Thus established cell strains consisted of pure epithelial cells (Fig. 2c and d).

Table 1 summarizes the results of a similar experiment. In each series of experiments the fate of cells cultured in the media corresponding to those described above was similar to that shown in Fig. 1. Only cells from 39-week-old rats (series 8), however, continued to grow in medium containing urea alone. In this case the start of growth, 10 weeks after inoculation, was delayed relative to that of cells cultured in medium containing both urea and a nitrosamine but a confluent monolayer gradually formed. Cells of all established strains listed in Table 2 had epithelial shape and orientation, and most of them had more than two relatively large nucleoli, compared with normal epithelial bladder cells which have one or two such nucleoli. Modal chromosome numbers of the cell strains varied from 70 to 80 except BES3P strain whose modal number was 42. The tendency of cells to pile up in a monolayer was rarely demonstrated.

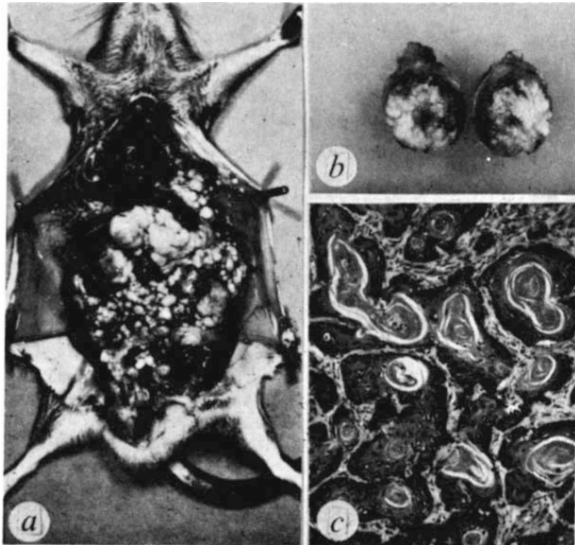


Fig. 3 Tumours developed from BES3B cells by intraperitoneal injection (a) and by intrabladder-wall injection (b), and histology of the tumour (c). (Haematoxylin and eosin, $\times 50$.)

Tumorigenicity of cells of the established strains was tested by injecting them into adult ACI/N male rats, some of which had been X irradiated on the previous day (Table 2). All cell strains induced in medium containing both urea and a nitrosamine and one strain in medium containing urea alone gave rise to tumours, after injection subcutaneously, intraperitoneally or into the wall of the bladder. In 1–3 months the tumours grew to a palpable size. All tumours contained areas of squamous metaplasia, histologically designated squamous cell carcinoma. Tumour cells were arranged in a cystic or papillary form, containing a keratin pearl (Fig. 3c). Intraperitoneal BES3B, BES8B or BES8P cells gave rise to many tumour nodules in the peritoneal cavity of recipients (Fig. 3a), some causing death. Tumours produced by injection of cells were serially transplantable into untreated syngenic rats.

Our results show that epithelial cells of the urinary bladder of adult rats transform into neoplastic cells when treated with urea combined with either BCPN or BBN. Because fibroblast-like cells did not grow, nitrosamine plus urea must

affect epithelial cells specifically. This recalls the *in vivo* carcinogenesis of the bladder when BBN or BCPN induced very few tumours originating from cells other than epithelial cells^{2,5}. Our experimental system is unique in the following ways. (1) The culture conditions reflect the *in vivo* condition for induction of bladder tumour by BBN—the concentration of nitrosamine in the medium is comparable with that of BCPN excreted in the urine when a carcinogenic concentration of BBN is administered in the drinking water (our unpublished data). Target cells are exposed continuously to nitrosamine as with *in vivo* carcinogenesis and urea is present in the medium. (2) Cell donors are adult animals and target cells come from the primary cultures, and not foetal or newborn animals as in previous experiments⁸. (3) Cells of the established strains develop cancers in untreated adult animals.

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Effect of split doses of X rays on neoplastic transformation of single cells

Using cloned hamster embryo cells in culture, we have found that if an X-ray dose is divided into two fractions, more neoplastic transformations occur than if the same dose is given in a single exposure. This could have important implications for radiation protection, where in general radiation is accumulated in a number of small fractions rather than in a single large exposure.

We used the *in vitro* system^{1,2}, in which primary or secondary cultures of hamster embryo cells are seeded at low density to allow for clone formation. More cells are seeded as the dose increases to account for cell killing. Twenty-four hours elapse between plating and irradiation, in order for cells to attach, after which cultures are incubated in a humidified atmosphere of 5% CO₂ in air for 9–10 d. They are then fixed, stained and examined for the presence of transformed clones, which can be distinguished morphologically from controls. The transformed cells show a loss of contact inhibition³, and pile up randomly, in contrast to normal cells^{1,2,4–6} (Fig. 1). We have described the relationship between the morphology of the transformed cells and their tumorigenicity *in vivo*^{1,2}; when injected into 2–3-week-old hamsters they give rise to fibrosarcomas (Fig. 2). Their ability to grow in semi-solid medium and in suspension, and their agglutinability by plant lectins have served as further criteria for transformation².

Table 1 Neoplastic transformation *in vitro* following single and split doses of X rays

Exp.	No. clones counted	Control Plating efficiency	Feeder present	No. transformed clones	Dose	No. clones counted	Irradiated Feeder present	No. transformed clones	% Transformed	Surviving fraction
1	6,000	3.2	+	0	75	2,081	+	14	0.675	0.89
					(37.5 × 2)	3,002	+	32	1.065	0.92
2	5,080	2.5	+	0	75	9,800	+	70	0.715	0.90
					(37.5 × 2)	8,430	+	95	1.129	0.94
3	3,002	2.0	+	0	75	2,120	+	10	0.494	0.90
					(37.5 × 2)	2,020	+	17	0.845	0.92
4	3,841	3.1	+	0	75	8,750	+	45	0.525	0.89
					(37.5 × 2)	7,300	+	77	1.050	0.93
	4,120	0.5	—	0	75	3,131	—	21	0.673	0.87
					(37.5 × 2)	3,010	—	34	1.121	0.90
5	4,210	2.2	+	0	50	3,010	+	6	0.199	0.95
					(25 × 2)	3,120	+	11	0.352	0.98
6	2,100	2.5	+	0	50	3,002	+	5	0.167	0.96
					(25 × 2)	3,502	+	9	0.254	0.99
7	5,751	4.1	+	0	50	5,125	+	10	0.195	0.90
					(25 × 2)	5,099	+	19	0.382	0.95
	4,106	0.5	—	0	50	3,135	—	6	0.192	0.88
					(25 × 2)	3,861	—	16	0.398	0.91

Using this model system, we have elucidated the dose-response relationship for transformation by single acute doses of X rays between 1 and 600 rad². The overall shape of the curve resembled that commonly observed for the production of tumours in experimental animals⁷, namely a rising transformation rate with doses from 1 to 150 rad, followed by a plateau between 150 and 300 rad, after which a further increase of dose resulted in a decrease in the proportion of colonies transformed. This decrease coincided with doses such that the number of

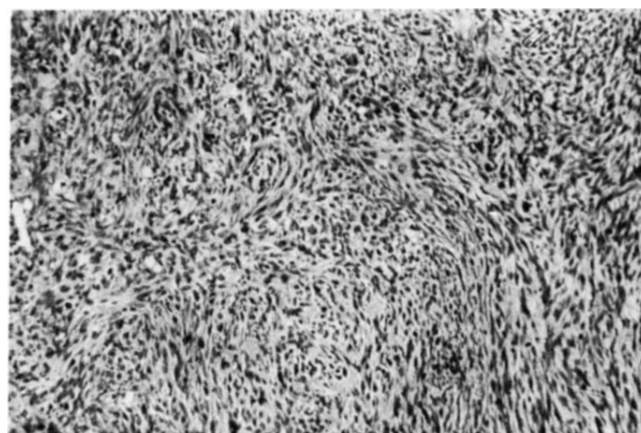


Fig. 2 Histological section of a non-invasive, non-metastasising fibrosarcoma growing in a 3-week-old hamster 10 d after a subcutaneous injection of 10^6 transformed cells obtained as follows. A transformed clone, of the type illustrated in Fig. 1c, produced by X irradiation of single cells, was isolated and grown up through 30 passages before inoculation (haematoxylin and eosin $\times 106$).

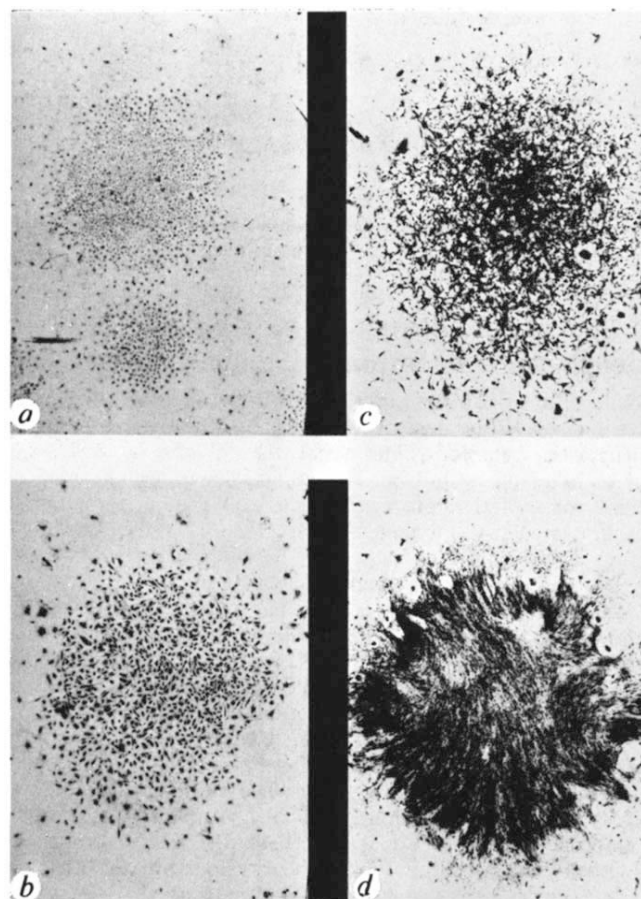


Fig. 1 *a* and *b*, 9-d-old clones of normal embryo cells (Giemsa $\times 10.6$); *c* and *d*, two representative 9-d-old clones of hamster embryo cells transformed *in vitro* by a split dose of 75 rad (Giemsa $\times 10.6$).

cells killed began to be significant. Our system inherently accounts for cell killing, however, because transformation is scored from among surviving colonies. Consequently, potentially transformable cells seemed more sensitive to the lethal effects of the accumulated damage at the higher doses.

We next pursued the problem of dose protraction since the problems of risk estimates for radiation are related to increments of low doses protracted over long periods, rather than a single acute exposure. For this reason, a single dose of 50 rad was compared with two doses of 25 rad 5 h apart. The doses were chosen to be on the ascending portion of the dose-response curve, while the time interval was a compromise, being long enough to allow for the repair of sublethal radiation damage, but short enough to minimise the probability of cell division between exposures. Plates receiving the single dose were exposed at a time mid-way between the delivery of the first and second doses to the plates. In later experiments the effect of a dose of 75 rad was compared with that of two doses of 37.5 rad 5 h apart.

The results are presented in detail in Table 1, summarised in Table 2 and plotted in Fig. 3. For both 50 and 75 rad, there is a clear increase in the proportion of transformed clones for doses delivered as two fractions compared with the same doses given as single exposures.

Table 2 Pooled data

	Clones counted	No. transformed	% Transformation
Control	38,210	—	—
75 rad	25,882	160	0.62
(37.5 × 2)	23,762	255	1.08
50 rad	14,272	27	0.19
(25 × 2) rad	15,582	55	0.35

To check whether the presence of feeder cells was essential for the production of transformed clones, some cells in these experiments were plated on feeder layers and others were not. While the feeder layer (at levels of 2×10^4 cells per 60-mm Petri dish) improved plating efficiency, it did not affect the proportion of cells transformed by the radiation.

At these low doses less than 10% of cells were killed, as can be seen from the surviving fractions listed in Table 1. It is also evident from Table 1 that more cells survived split doses than single doses, which shows that sublethal damage was repaired, as in almost every biological system studied.

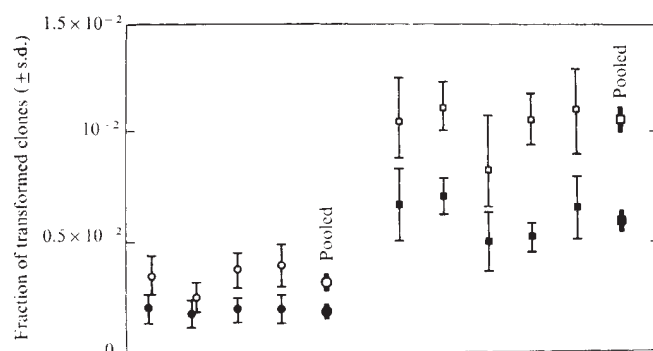


Fig. 3 Incidence of cell transformation in cloned cultures of hamster embryo cells. Four replicate experiments were performed in which the effect of a single dose of 50 rad (●) was compared with that of two doses of 25 rad (○). The results of the individual experiments are shown, together with the pooled data; vertical bars are standard deviations. The consequence of a single dose of 75 rad (■) compared with two doses of 37.5 rad (□) are shown.

The results suggest that even at these low doses there is a strong correlation between the proportions of cells killed and transformed. When a dose of radiation is delivered as two fractions fewer cells receive lethal injury, and therefore more cells sustain sublethal damage than when the same dose is delivered in a single exposure. Since X-ray transformation presumably involves sublethal events, it is not surprising that splitting the dose leads to less killing but enhanced transformation.

Comparison of our *in vitro* data with similar studies of tumour induction *in vivo* is interesting, although the latter show no clear-cut pattern. Most studies of tumour induction in animals have involved larger doses and longer time intervals than used in our study, and leukaemias and lymphomas rather than solid tumours. Kaplan and Brown^{8,9} found that 475 r., given to mice as four exposures at 4 or 8-d intervals, was more tumorigenic than the same dose given as a single exposure or in daily fractions. By contrast, Metalli *et al.*¹⁰ concluded that split doses of sublethal irradiation with short fractionation intervals are less efficient inducers of tumours and leukaemias than a single dose. Upton *et al.* found a complex pattern of X-ray-induced myeloid leukaemia in mice¹¹ that was intermediate between these two sets of results.

Our demonstration of an increased incidence of transformation when the dose is divided, albeit with an *in vitro* system, is

germane to the problems of radiation protection. When radiation workers and the general public are exposed to ionising radiation it is usually in the form of multiple small doses rather than a single large dose, as commonly used for experimental studies with small animals. For most end-points observed, including cell killing, fractionation reduces the effect of a given dose of radiation, but if the opposite is true for transformation ours will be an important observation in practical terms.

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Association of polyanion resistance with tumorigenicity and other properties in BHK/21 cells

QUIESCENT, non-growing cultures of normal fibroblasts may arise as a result of absence of anchorage^{1,2} or deprivation either of serum macromolecules³⁻⁵ or of a variety of nutrients⁶. This state can also be induced by sulphated polysaccharides^{7,8} and we have reported the inhibition of growth of normal BHK/21 hamster fibroblasts in monolayer by dextran sulphate and heparin⁹ and the protection against this inhibition by purines¹⁰. Goto *et al.* have also found that dextran sulphate added to growing cultures markedly reduces the saturation density of established lines not transformed by viruses, but not that of primary embryonic fibroblasts¹¹. The ability of many cells to grow in agar only after transformation with tumour viruses has been similarly ascribed to the inhibitory effects of sulphated polysaccharides^{8,12} and a close quantitative relationship has been reported between tumorigenicity and plating efficiency in agar¹³.

In the light of these findings and the known existence of sulphated polysaccharides and proteoglycans in connective tissue, it is appropriate to seek a role for such polyanions in growth control in such tissues. We have obtained dextran sulphate resistant cultures of normal hamster BHK/21 cells and studied their properties. The results show that there is a close correlation between resistance of clonal sublines to dextran sulphate and a number of other properties: high

Table 1 Clonal variants of BHK/21 cells (see text) compared with parent untransformed and transformed with polyoma virus (PY)

Inhibitory cells	TD ₅₀	Inhibitional concentration dextran sulphate (μg ml ⁻¹)	Serum dependence	Attachment	Orientation
BHK/21	3 × 10 ⁴ –10 ⁵	< 3	+	+	+
'S'-type variant	3 × 10 ⁴ (1)	3 (1)	+	+	+
'I'-type variant	10 ² –10 ³ (12)	4–32 (8)	+	+	±*
'R'-type variant	10 ² (9)	> 100 (24)	—	±†	—
PY BHK/21	10 ²	> 100	—‡	+	—

Figures in brackets refer to number of sublines tested. Tumorigenicity was tested by injecting 0.5 ml cell suspension subcutaneously into young adult hamsters (four animals per suspension) containing 10², 10³, 10⁴, 10⁵ cells; animals were observed for 5 months. The inhibitory concentration of dextran sulphate was that which prevented the doubling of cells in 4 d (Fig. 1). Serum independence is the ability to grow continuously in serum-free HEMP medium (E4, 4 volumes; Hams F12, 1 volume; Pirls, 1 volume); methocel¹⁰ (4,000 c.p.s. Dow Chemical Corp.) to 0.2%; dextran sulphate to 10 μg ml⁻¹.

*Cells pile up in the centre of large colonies, but show orientation at periphery.

†Cells attach initially but later grow in suspension.

‡Clarke, G. D., and Ryan, P. R. (unpublished) and Bush¹⁸.

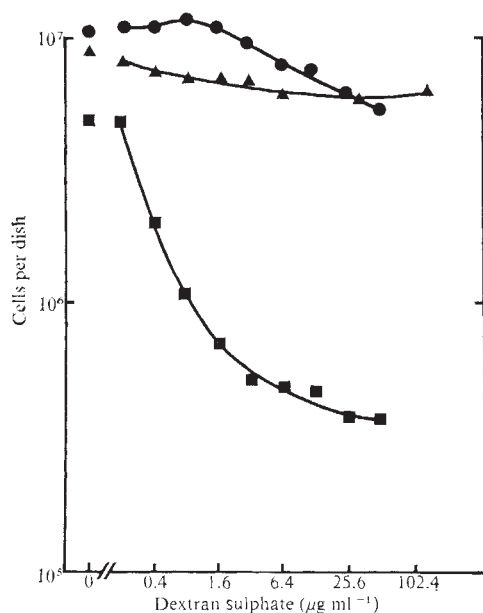


Fig. 1 Cells were grown in duplicate dishes of E4 medium containing 5% serum from an inoculum of 3×10^5 per 5 cm dish at 37° C for 4 d. After removal with trypsin versene they were counted with a Coulter Electronic Counter. ■, Original BHK/21 cells; ●, BHK/21 cells transformed with polyoma virus and, ▲, clonal subline 'R'-type'. Points are means of duplicates.

tumorigenicity, serum independence and growth in suspension.

While the growth of normal BHK/21 cells is completely inhibited by 2 μg ml⁻¹ dextran sulphate, that of polyoma transformed cells is only marginally affected by 50 μg ml⁻¹ (Fig. 1).

To select for resistant variants, therefore, normal cells were seeded at 3×10^5 per dish in medium containing 5% serum and 0.5 μg ml⁻¹ dextran sulphate. Dishes were incubated at 37° C until a dense culture was obtained. Subculture was then made at one-tenth dilution into both 0.5 and 1.0 μg ml⁻¹ dextran sulphate. This regime of subculture at twofold higher concentrations was continued reverting to the same concentration only when inadequate growth was obtained at the higher, until cells were growing vigorously at 16 μg ml⁻¹ of dextran sulphate. Growth was continued in this medium with progressively decreasing inocula until cells grew reproducibly from 10⁴ per 9 cm dish. This complete selection process was repeated three times and, on two occasions, the cells were then cloned in dextran sulphate-free medium with 10% serum (plating efficiency 25–30%). In the course of each selection it was noted that giant cells appeared, most of which floated or remained loosely attached to the dish; aliquots from the supernatant were always, therefore, incorporated in subcultures. After a few pas-

sages in dextran sulphate the cells lost the distinctive oriented appearance of BHK/21 and, in later passages, many remained rounded with apparently healthy cells appearing in suspension. This behaviour was also shown in dextran-free medium. From the resistant cells colonies produced were classified in three groups. Most colonies were composed of spread cells initially and showed central piling up. Sublines from such clones ('I'-type') showed variable resistance to dextran sulphate and 10²–10³ cells injected into hamsters formed tumours in 50% of animals (TD₅₀). One colony resembled the original cells, showing oriented growth and full sensitivity to dextran sulphate ('S'-type').

Table 2 Interaction of BHK/21 cells and dextran sulphate

Dextran sulphate in medium A (μg ml ⁻¹)	Serum in medium B (%)	% Labelled nuclei
0	0	2
0	3	53
20	3	32
200	3	22
2,000	3	15

BHK/21 cells seeded at 3×10^5 cells per 5 cm dish in E4 medium containing 0.5% serum and incubated for 3 d. Medium was removed, duplicate dishes treated at room temperature for 1 min with either E4 or E4 + dextran sulphate (Medium A). Dishes were then washed with E4 (two, three and four washes were used and results were not significantly different) and incubated for 20 h with E4 medium or E4 + 3% serum (Medium B) and ³H-thymidine at 1 μCi ml⁻¹. Following autoradiography the percentage of labelled nuclei was calculated from 300 in each of four dishes per set.

Nine colonies ('R'-type') consisted almost entirely of rounded cells and sublines from these all had a TD₅₀ as low as that of polyoma transformed BHK/21 cells and were also as resistant to dextran sulphate (Table 1 and Fig. 1). It was further found that all nine sublines were capable of continuous propagation without serum in a complete synthetic medium. A further 15 'R'-type' sublines were established from clones arising in medium containing 16 μg ml⁻¹ of dextran sulphate (plating efficiency 0.5–2%) all of which grew continuously in serum-free medium. Temin suggested that sulphated polysaccharides might act by combining with serum growth factors and indeed a small precipitate containing protein occurs in dextran sulphate medium and adheres to the dish (R. C. Hallows, unpublished). Our results, however, indicate that quiescent BHK/21 cells can also react with dextran sulphate in the absence of serum in a manner that, even after thorough washing, inhibits their response to serum (Table 2), indicating that it also binds to the cells.

In view of the tendency of dextran sulphate resistant cells to grow in suspension and the observation of floating cells in dextran sulphate containing medium some hours after seeding, the effect of the polyanion on attachment of cells to dishes was

Table 3 Attachment of BHK/21 and PY cells in dextran sulphate

Serum %	Dextran sulphate ($\mu\text{g ml}^{-1}$)	Cells attached (%)	
		BHK	PY BHK
0	0	97	98
0	4	97	97
0	16	98	96
0	64	97	97
5	0	83	71
5	4	36	14
5	16	35	2
5	64	32	1

Cells were removed from a culture in E4 medium containing 20% calf serum with 0.05% trypsin (Difco 1:250) in 0.1% versene, washed with medium and resuspended in the same medium containing only 5 mM NaHCO_3 and with 30 mM HEPES buffer adjusted to pH 7.0. Aliquots of the same medium (5 ml) were prepared with serum and dextran sulphate as indicated and warmed to 37° C. Cells were seeded into these at 6×10^5 each and 2.5 ml of each suspension dispersed into duplicate dishes in a 37° C room. After 20 min the supernatant medium was gently poured off into plastic pots. The dishes were then gently washed with a further 2.5 ml of medium containing 10% serum and the washings added to the pots. Cell numbers were counted in each suspension with a Coulter Electronic Counter and the numbers attached calculated.

examined; Table 3 shows that the attachment of both normal and transformed cells is inhibited in the presence of serum. This behaviour is in line with the observation that mouse L cells in monolayer culture detach from the dish within 10 min on the addition of dextran sulphate¹⁴.

It is premature to attempt a detailed understanding of the mechanism of inhibition by, and resistance to, dextran sulphate. The simplest hypothesis, that polyanions by competition for cell attachment sites inhibit anchorage which is essential for growth of normal but not transformed cells, is untenable. The inhibition of attachment of BHK/21 cells by dextran sulphate in short term experiments described in Table 3 demonstrates a delay but within a few hours the majority of cells are attached and spread. Moreover, in the absence of polyanions, such cells can grow in suspension.

Our observations with these variants suggest that resistance to dextran sulphate is correlated with a tendency to grow in suspension, independence of serum growth factors and high tumorigenicity. We postulate that dextran sulphate, which is known to react with many proteins¹⁵, binds to the cell membrane so as to block the interaction with the macromolecular growth factors in serum and to block temporarily the interaction with the solid substrate. The membrane functions involved are probably required for the growth of normal but not that of transformed fibroblasts. The selection process in dextran sulphate under which resistant cells emerge may thus be considered as comparable with that under which malignancy emerges *in vivo*. It is not yet clear whether the process is analogous, namely whether sulphated polyanions of connective tissue mediate the control of cell proliferation in those tissues. Meanwhile it is important to know whether the phenomena reported here can be observed also in other cell systems.

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Antibody-dependent cell-mediated damage to schistosomula *in vitro*

ANTIBODY-DEPENDENT cell-mediated cytotoxic effects have been described in many *in vitro* models involving single-cell suspensions of mammalian or avian target cells, and could be important in the rejection of tumours and allografts^{1–6}. Similar responses could also be involved in protective immunity against metazoan parasites, and evidence for cell-mediated effector mechanisms of various types has emerged from work on *Nippostrongylus braziliensis*⁷ and *Litomosoides carinii*⁸ in rats. Here we present two new aspects of cell-mediated responses to metazoan parasites. First, we have adapted the ⁵¹Cr-release technique to assay damage to schistosomula of *Schistosoma mansoni* *in vitro*. Second, we provide evidence that normal human peripheral blood leukocytes, in the presence of sera from patients infected with *S. mansoni*, can damage labelled schistosomula in the absence of added complement.

A naturally-infected field population of *Biomphalaria pfeifferi* was used to initiate a laboratory colony of *S. mansoni*, which was subsequently maintained in locally collected snails and Swiss albino outbred mice. Schistosomula were prepared from cercariae by a modified skin penetration technique⁹, and were stored at 4° C overnight in Hanks' balanced salt solution containing antibiotics and lactalbumin hydrolysate 0.5%, together with 10% heat-inactivated normal baboon serum. This storage procedure was not associated with a detectable loss of schistosomula or decrease in viability, as judged by motility and ability to metabolise fluorescein dibutyrate¹⁰. After overnight storage, most of the supernatant fluid was removed, and the organisms, after resuspension in the residual supernatant, were labelled with ⁵¹Cr-sodium chromate (Radiochemical Centre, Amersham). Incubation of 10⁴ organisms with 0.5 to 1 mCi of ⁵¹Cr in a volume of less than 0.5 ml for 3–4 h at 37° C gave count rates of 50–300 c.p.s. per 100 organisms. Labelled schistosomula were washed five times in HEPES-buffered Eagle's minimal essential medium (MEM) with antibiotics, and were resuspended at 500 or 1,000 organisms per ml in MEM containing 10% heat-inactivated foetal calf serum (MEM/FCS).

Serum samples were obtained from patients with a long history of presumptive exposure to *S. mansoni* and with eggs detectable in varying numbers in the faeces by the Kato technique¹¹. Control samples were taken from laboratory workers with no known exposure to *S. mansoni*. All sera were heat-inactivated and were diluted in MEM/FCS before testing. Peripheral blood leukocytes were prepared from uninfected human subjects by defibrination and centrifugation over Ficoll-Hypaque¹². Interface cells were washed twice in MEM and resuspended in MEM/FCS at varying cell concentrations. Such preparations contained both polymorphonuclear and mononuclear leukocytes.

The cytotoxicity test was carried out in a total volume of 0.3 ml in 38 × 7 mm sterile plastic tubes, incubated in humidified plastic boxes. At the end of the incubation period, 0.15 ml of the

Table 1 Release of ^{51}Cr from schistosomula after incubation with various sera in the presence or absence of normal human peripheral blood leukocytes

Serum dilution†	Isotope release (%)*					
	With serum + cells†			With serum alone‡		
	1 : 6	1 : 24	1 : 96	1 : 6	1 : 24	1 : 96
Serum						
A40/1 infected	54	48	39	24	22	24
A39/1 infected	51	49	44	26	26	22
A39/4 infected	50	51	48	25	23	22
A38/3 infected	53	49	43	26	23	25
AEB control	28	29	24	23	23	24
LTW control	27	27	27	23	23	23
SN control	26	28	27	22	24	21
GK control	29	28	25	22	23	23
Medium§ —	—	27	—	—	24	—

Statistical summary: analysis of variance, 'cells' $\times$ 'serum' interaction, $P < 0.001$ ($F = 43.4$; 8 and 162 d.f.); multiple range tests: Values in italics are significantly different from the 'medium with cells' control ($P < 0.05$).

* Measured after 14 hours.

† Schistosomula were cultured either with or without effector cells at a multiplicity of 2,000 : 1.

‡ Final dilution of serum in the cytotoxicity assay mixture.

§ Schistosomula cultured with or without effector cells, without added serum.

supernatant was removed, and both cell and supernatant tubes were counted for ^{51}Cr . Four replicates for each group were set up, and results are expressed as the geometric mean of the original percentage isotope release values. The logarithmically-transformed data were tested by multifactorial analysis of variance followed by Duncan's multiple range tests¹², and a summary of the statistical analysis is given for each set of data.

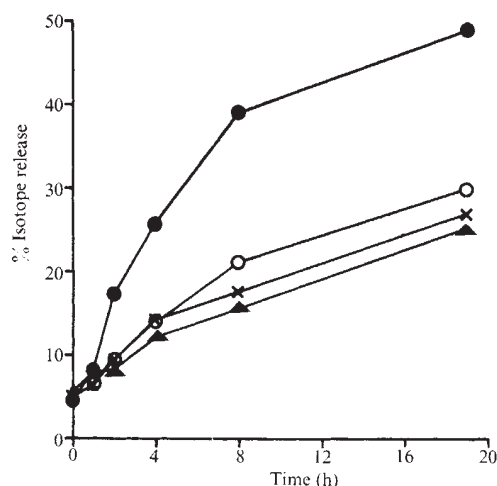


Fig. 1 ^{51}Cr -labelled schistosomula were incubated for varying periods of time in the presence of: ▲, medium alone; x, serum alone (A39/1 at 1/30 final concentration); ○, cells alone (multiplicity of 2,000:1); ●, cells + serum. Analysis of variance: 'effector preparation' $\times$ 'time' interaction, $P < 0.001$ ($F = 4.54$; 15 and 72 degrees of freedom). Multiple range tests: 'Cells + serum' different from other preparations at 2, 4, 8 and 19 h ($P < 0.05$); 'Cells alone' different from 'medium alone' at 8 h ($P < 0.05$).

The time course of isotope release in the presence of various combinations of serum from an infected patient and normal cells is shown in Fig. 1. Release in the presence of medium alone was slow, amounting to only 25% after 19 h. Antibody alone, at a final concentration of 1 : 30, caused no significant increase in isotope release. Cells alone, at a multiplicity of 2,000 : 1 to the target organisms, caused a slight increase in isotope release. This multiplicity was chosen arbitrarily, and it should be borne in mind that the target is a large multicellular organism measuring approximately $400 \times 100 \mu\text{m}$. In later experiments, significant cytotoxicity by cells and antibody was found at multiplicities down to 100 : 1. Cells in combination with antibody caused a marked increase in isotope release which was

significantly different from the effect of cells alone from 2 h of incubation onwards. By 19 h, isotope release in the presence of cells and antibody was beginning to show a plateau effect at approximately 50%, and in separate experiments it was observed that only 50–60% of the total isotope incorporated could be released by repeated freezing and thawing in hypotonic solution, a procedure which killed 100% of the schistosomula. This presumably reflects the multicellular nature of the organism, which may be resistant to complete disruption. The relationship between isotope release and death of the organism was difficult to determine, but incubation of various preparations of schistosomula with fluorescein dibutyrate¹⁰ and subsequent examination by fluorescence microscopy showed that the percentage of isotope released in a preparation was approximately related to the proportion of organisms that were immobile and that failed to metabolise the fluorescein ester.

The data in Fig. 1 were derived from a single serum sample at a single dilution. Table 1 shows the results of an experiment in which four sera from infected subjects and four sera from control subjects were tested at three dilutions. In separate experiments, a total of eleven test sera and four control sera were examined. None of the sera was toxic for schistosomula in the absence of added leukocytes. All the test sera showed significant effects in the presence of added cells, in seven cases at dilutions down to 1 : 96. Later examination of three of the positive sera (A40/1, A38/3 and A39/1) showed that significant effects could be obtained at dilutions down to 1 : 320. None of the control sera showed a significant effect, even at concentrations as high as 1 : 6.

These results indicate that schistosomula can be damaged *in vitro* by normal human peripheral blood leukocytes together with sera from infected patients, but not with sera from control subjects. Unlike "lethal antibody"⁹ and granulocyte-mediated damage to opsonised organisms¹³, both of which depend on the presence of large amounts of added complement, the effect described here is complement-independent, in that heat-inactivated sera were used throughout. An analysis of the antibody and cell types responsible for eliciting the effect will be published elsewhere (A. E. B., unpublished); preliminary observations indicate that maximum cytotoxic activity is associated with an eosinophil-rich polymorphonuclear leukocyte fraction.

The relevance of the observations to resistance to reinfection cannot at present be established, particularly as it has not yet been demonstrated clearly that human patients do develop an effective immunity to *S. mansoni*. Recent evidence in experimental schistosomiasis in rats, however, has shown that resistance to infection can be transferred to normal animals by injection of immune serum, but that irradiation of immune rats

on the day of cercarial challenge abolishes immunity¹⁴. These findings suggest that both antibody and a radiosensitive cell may be involved in resistance in rats. Our results indicate that a similar mechanism could be effective in man, and provide a technique for analysing the response in more detail.

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C3 activation and T-independent B cell stimulation

SOME highly polymerised immunogens with repeated antigenic determinants, such as bacterial polysaccharides or polymerised flagellin and hapten conjugates of these, can stimulate mouse B lymphocytes to make antibody without requiring the cooperative helper activity of T lymphocytes¹. The mechanism whereby such T-independent immunogens, after interaction with the surface receptors on specifically responsive B lymphocytes, trigger these into activity is uncertain. Dukor and Hartmann² have postulated that binding of activated C3 to complement receptors on B cells acts as a necessary second signal for antibody production. They envisage that T-independent antigens and B-cell mitogens generate this second signal by their ability to activate C3 through the 'bypass' pathway, whereas in the case of T-dependent antigens the signal derives from C3 cleavage by proteases released by activated T cells.

Recent work in our laboratory suggests that neither high epitope density³ nor polyclonal mitogenicity¹⁷ are adequate explanations for T cell independent immune induction. Here we extend these observations by examining the correlation between the immunogenic properties of various antigens tested *in vivo* and their capacity to activate mouse C3 *in vitro*. On the basis of the above model, all T-independent antigens should be able to activate C3.

Fresh C3H/He mouse serum was used as a source of C3 and the origins of the antigens are listed in Table 1. Sheep anti-mouse

C3 serum was made by a modification of the method of Mardiney and Müller-Eberhard¹². For C3 cleavage, 0.05 ml of the test sample in saline was added to 0.1 ml of fresh mouse serum diluted with an equal volume of Veronal buffered saline¹² containing 0.025 M MgCl₂ and 0.025 M ethyleneglycol bis-(aminoethyl)-tetraacetic acid (EGTA) (Koch-Light). The mixture was incubated at 37° C for 45 min. The presence of cleavage products was detected by antigen-antibody crossed electrophoresis¹⁴ in agarose gel containing 0.01 M EDTA and sheep anti-mouse C3 serum.

The presence of EGTA and Mg²⁺ during incubation provided conditions for activation of complement by the 'bypass' mechanism only^{15,16}, and excluded any activation by the Ca²⁺-dependent classical pathway because of pre-existing antibody reacting with the antigen. The sensitivity of the method for detecting activation of C3 was tested using various concentrations of *Escherichia coli* 0.55:B5 lipopolysaccharide (LPS) (Difco) and levan (Fig. 1). LPS was detectably active at concentrations down to 2 µg ml⁻¹ (although this is not visible in the photograph) and obvious activation was obtained at 4 µg ml⁻¹; the preparation of levan was clearly active at 1 µg ml⁻¹ and some activation was detected at the lowest concentration tested (0.5 µg ml⁻¹).

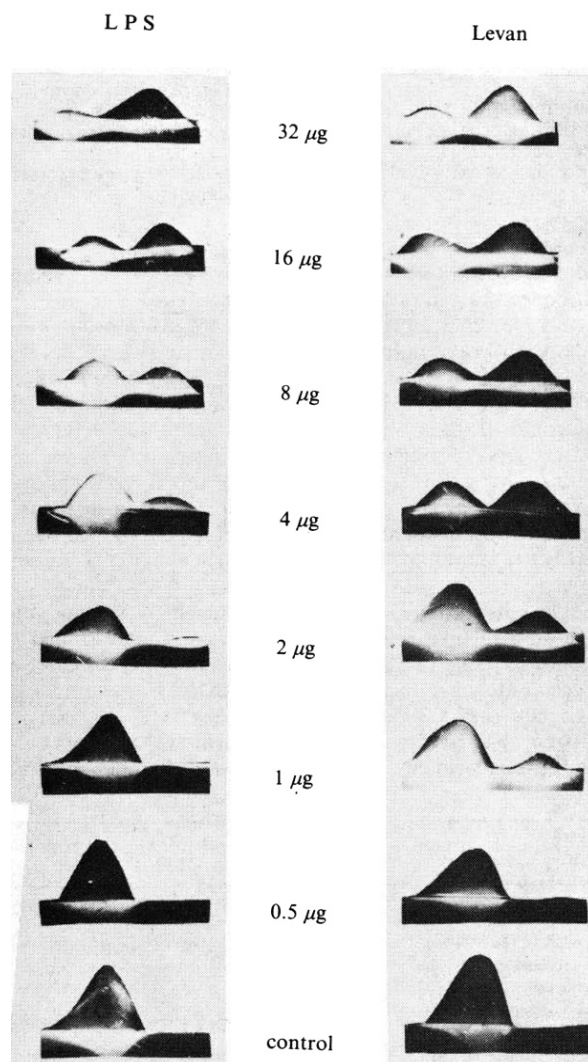


Fig. 1 Comparison by antigen-antibody crossed electrophoresis of C3 activation by various doses of *E. coli* 0.55:B5 LPS and levan. The left hand peak is native C3, the right hand (faster moving) its cleaved product (anode on the right).

Table 1 C3-activating properties of antigens and mitogens

Antigens and mitogens tested including origin	Concentration tested ($\mu\text{g ml}^{-1}$)	C3 activation
LPS <i>E.coli</i> 0.111:B4 (Difco)	400	+
LPS <i>E.coli</i> 0.55:B5 (Difco)	400	+
	200	+
Acid hydrolysed LPS <i>E.coli</i> 0.55:B5 (ref. 4)	400	—
Alkali hydrolysed LPS <i>E.coli</i> 0.55:B5 (ref. 4)	400	—
Hydroxylaminolysed LPS <i>E.coli</i> 0.55:B5 (ref. 5)	400	—
LPS <i>Salmonella</i> mR 345 (ref. 6)	400	—
LPS <i>Salmonella</i> TV-119 (ref. 6)	400	+
LPS <i>Serratia marcescens</i> (ref. 7)	400	+
LPS <i>Shigella shigae</i> (ref. 8)	400	+
*CoF	10	+
	5	+
†CoF	5 units	+
	1 unit	+
‡SIII	400	—
§SIII	1,000	—
	400	—
Levan (from <i>Aerobacter levanicum</i>) (ref. 11)	400	+
	10	+
DNP-lys-SIII (ref. 10)	400	—
DNP-lys-levan (ref. 11)	400	+
	10	+
¶Hyaluronic acid (Hy)	400	—
¶¶DNP-lys-Hy	400	—
¶¶Polyglutamic acid (PGA)	400	—
¶¶DNP-lys-PGA	400	—
DNP ₅₀ BSA (ref. 3)	400	—
DNP ₅₀ BSA (ref. 3)	400	+
	10	+

*Cobra venom factor was prepared from *Naja naja* (ref. 9).

†Commercial anticomplementary factor from cobra venom (Cordis Laboratories).

‡Pneumococcal polysaccharide from Type III pneumococci (Wellcome, Lot K 5045).

§Pneumococcal polysaccharide from Type III pneumococci (ref. 10).

||Preparations shown to behave as T-independent antigens.

¶Ref. 17.

Most of the antigens and mitogens were tested at a relatively high concentration (400 $\mu\text{g ml}^{-1}$ of incubated mixture). Some were also tested at lower and higher concentrations as shown in Table 1. Cobra venom factor (CoF), various preparations of LPS (except from *Salmonella* mR 345), levan and DNP₅₀BSA activated C3. Type III pneumococcal polysaccharide (SIII), hyaluronic acid, polyglutamic acid and DNP₅₀BSA were inactive. DNP conjugates of various polymers (which are all immunogenic with respect to the DNP moiety^{11,17}) behaved in the same way as the polymers themselves. Treatment of LPS with mild acid or alkali which abolishes its mitogenicity (G.G.B.K., unpublished) also abolished its C3 activating properties.

Table 2 lists the C3 activation produced by various antigen preparations whose mitogenic activity has been examined in the presence of foetal calf serum¹⁷. There is no apparent correlation between mitogenic and C3 cleaving activity. For example, levan which activates C3 very efficiently (Fig. 1) is a less potent 'mitogen' than SIII which did not activate C3 (Table 1).

In these experiments the effect of various T-independent anti-

Table 2 Comparison of C3-activating and mitogenic properties of various polymers

	C3 activation	Mitogenicity* ³ H-thymidine uptake (c.p.m. $\times 10^{-4}$ ml ⁻¹)
LPS <i>S.marcescens</i>	+	5.6
LPS <i>E.coli</i>	+	5.3
SIII	—	1.36
Levan	+	0.72
Hyaluronic acid	—	1.32
Polyglutamic acid	—	0.52
Negative control	—	0.32

*Cell proliferation in normal mouse spleen cell cultures induced by optimal stimulatory doses, and assayed by uptake of ³H-thymidine; cells grown in serum-containing medium (data taken from ref. 17).

gens on mouse C3 was examined, as their immunogenic and mitogenic properties have been previously tested in this species. Earlier observations that LPS and some other polysaccharides activate C3 by the 'bypass' mechanism in various species¹⁸⁻²⁰ are confirmed.

Regarding the significance of C3 activation for T-cell independent B cell triggering however, two points may be made. First, the capacity of an antigen to activate C3 does not necessarily make it T independent. For example, Klaus and Cross³ have shown that DNP₅₀BSA elicits a T-cell dependent IgM response; yet DNP₅₀BSA activates C3 effectively (Table 1). Furthermore, the C3 activating CoF itself, which readily elicits antibody in intact mice (and for that reason normally becomes ineffective within 4-5 d) has a prolonged action and fails to elicit detectable antibody in thymus deprived mice (J.P. and J.H.H., unpublished).

Second, the capacity to activate C3 by the 'bypass' mechanism, at least as measured by cleavage of C3 *in vitro*, does not seem to be an obligatory feature of T-independent immunogenicity (Table 1). Nevertheless our preparation of DNP-lys-levan, which activates C3, is considerably more immunogenic in C3H mice than DNP-lys-SIII, which does not. Similarly, native LPS is a more potent immunogen than LPS treated with acid or alkali (G.G.B.K., unpublished). (In the latter case, however, the comparison is complicated by the fact that native LPS is a potent polyclonal mitogen.)

It seems that neither activation of fluid phase C3 nor 'non-specific' (mitogenic) activation¹⁷ can be essential prerequisites for specific B cell triggering. The capacity of an antigen to activate fluid phase C3 and/or to cause polyclonal B-cell proliferation, however, may well enhance its intrinsic immunogenicity. Furthermore, it is possible that T-independent antigens which do not activate fluid phase C3 may nevertheless do so, when bound at a cell surface. This possibility is being investigated.

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Differential recognition by thymocytes of H-2 and non-H-2 alloantigens

In this report we examine the behaviour of mouse thymocytes in the mixed lymphocyte culture. The thymus is believed to contain large numbers of T cell precursors or immature T cells undergoing differentiation before migrating to the peripheral lymphoid areas as mature, immunocompetent cells. Our results suggest that the thymus contains a class of cell possessing the potential to react against products of H-2 and non-H-2 loci, but that mixed leukocyte culture reactivity against H-2 depends on a factor(s) present in normal mouse serum.

Thymus-dependent (T) lymphocytes have long been suspected of playing a major role in cytotoxic reactions against cell-associated alloantigens¹⁻⁵. T-cell mediated cytotoxicity is usually dissociated into two phases: a recognition phase, resulting in clonal proliferation of antigen-activated cells, and a destructive phase, in which activated cells within the responding population demonstrate cytolytic activity against cells bearing the sensitising antigens⁶⁻⁹. These proliferative and effector phases have been studied *in vitro* using the mixed leukocyte culture (MLC) and cell-mediated lympholysis (CML) assays, respectively. For most species MLC activation as well as cell destruction are associated with loci of the major histocompatibility complex (MHC). In the mouse, however, MLC activation can be induced by genetic differences at loci segregating independently of the MHC (H-2)^{10,11}.

Lymphocyte activation was examined in the sensitive miniaturised mouse MLC assay¹² using serum-free¹³ and mouse

serum supplemented¹⁴ culture media. The genetics of mouse strains used in this study have been described elsewhere¹⁵⁻¹⁸.

MLC responsiveness of thymocytes from 3-5-week-old mice against H-2 and non-H-2 alloantigens was determined in serum-free and mouse serum supplemented media. Figure 1 shows thymidine (³H-TdR) incorporation for C57BL/10 (H-2^b) thymocytes stimulated with X-irradiated spleen cells from (1) B10.A (H-2^a) or B10.D2 (H-2^d), two strains possessing genetic differences with C57BL/10 at H-2, and (2) A/Jax (H-2^a) or DBA/2 (H-2^d), two strains differing from C57BL/10 at both H-2 and non-H-2 loci. C57BL/10 thymocytes showed no MLC reactivity against either B10.A or B10.D2 spleen cells in the absence of mouse serum; however, in the presence of serum strong MLC activation occurred. In contrast, C57BL/10 thymocytes responded against A/Jax and DBA/2 spleen cells in both culture media. Similar response patterns have been obtained using numerous strain combinations. Reactions of B10.BR thymocytes mixed with X-irradiated spleen cells from strains with various genetic differences are shown in Table 1. That non-H-2 differences alone are sufficient to induce MLC activation in serum-free medium is demonstrated by the B10.BR-C58 combination.

This differential reactivity of murine leukocytes responding against H-2 and non-H-2 alloantigens in serum-free and mouse serum supplemented media can be demonstrated with thymocytes from 3-5-week-old mice, but is not demonstrable with cells from peripheral blood, lymph node, spleen, or thymus of cortisone-treated mice. These tissues contain few immature cells and are reactive against H-2 alloantigens in either serum-free or serum supplemented medium.

Recent reports^{19,20} have shown that various agents—thymus extracts or cyclic AMP—cause differentiation of presumably immature thymocytes into functionally mature T cells. A similar mechanism might also be responsible for the serum requirement in thymocyte responses against H-2 alloantigens. One approach in the examination of this point was to use serum from homozygous nude mice which are athymic and therefore should be deficient in thymus hormone(s). The activity of serum from nude mice (possessing the homozygous *nu* locus on a BALB/c background) was compared with serum from normal BALB/c mice (Fig. 2). B10.D2 spleen cells or thymocytes were mixed with X-irradiated spleen cells from DBA/2, a strain possessing only non-H-2 differences, or C57BL/10, a strain possessing an H-2 difference, in media supplemented with serum from either normal or homozygous nude mice. The results indicate that B10.D2 thymocytes (Fig. 2a) respond much more strongly against C57BL/10 spleen cells (an H-2 difference) in medium supplemented with serum from normal BALB/c mice than in medium supplemented with serum from nude mice. In contrast, thymocyte responses against the non-H-2 antigens (DBA/2) showed no marked difference in the ability of the two sera to support the mixed reactions. There was little or no difference between the ³H-TdR incorporation by spleen cells responding against H-2 or non-H-2 alloantigens in the two sera (Fig. 2b). These results parallel the serum requirement for MLC activation of thymocytes with H-2 but not non-H-2 alloantigens shown in Figure 1 and Table 1. Note, however,

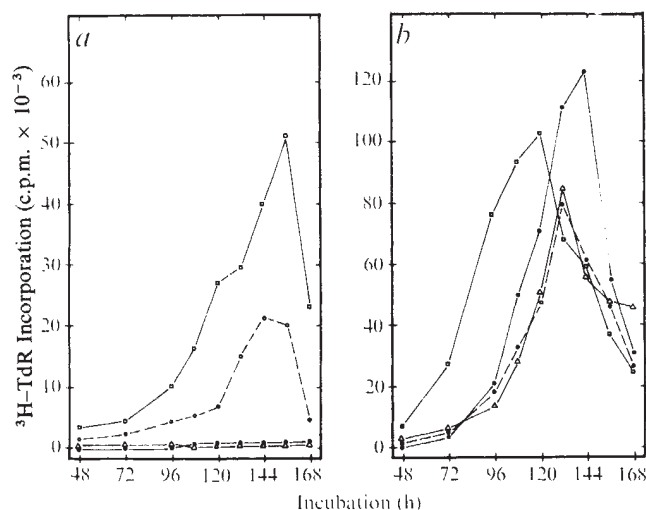


Fig. 1 MLC activation of thymocytes by H-2 and non-H-2 alloantigens in serum-free and mouse serum supplemented media. C57BL/10 thymocytes were mixed with X-irradiated B10.A (Δ), B10.D2 ($\bullet$), A/Jax ($\circ$) or DBA/2 ($\square$) spleen cells in serum-free (a) or mouse serum supplemented (b) media. 2.0 μ Ci ³H-TdR were added to each culture 8 h before termination of MLC at the times indicated.

Table 1 MLC responses of normal thymocytes against H-2 and non-H-2 alloantigens

Responding strain	Stimulating strain	Genetic difference in MLC combination	Minus serum		Plus serum	
			Mean c.p.m. $\pm$ s.d.		Mean c.p.m. $\pm$ s.d.	
B10.BR	B10.BR	None	547 $\pm$ 68		315 $\pm$ 23	
	C58	non-H-2	4,318 $\pm$ 65		61,748 $\pm$ 8,134	
	B10.A	H-2	782 $\pm$ 600		711 $\pm$ 340	
	A/Thy-1	H-2, non-H-2	2,538 $\pm$ 51		591 $\pm$ 103	
	B10.D2	H-2	528 $\pm$ 23		105,692 $\pm$ 2,819	
	DBA/2	H-2, non-H-2	12,109 $\pm$ 2,295		220,766 $\pm$ 19,470	

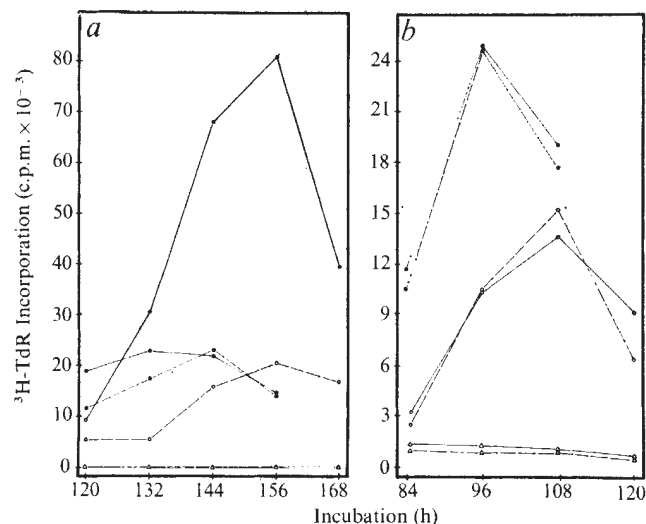


Fig. 2 MLC activation of thymocytes and spleen cells by H-2 and non-H-2 alloantigens in medium supplemented with serum from normal or nude mice. B10.D2 thymocytes (a) or spleen cells (b) were stimulated with X-irradiated C57BL/10 (○) or DBA/2 (●) spleen cells in medium supplemented with serum from normal (—) or nude (---) mice. 2.0 μ Ci 3 H-TdR were added to each culture 8 h before termination of MLC at the times indicated.

that some reactivity occurs using the serum from nude mice in the reaction of B10.D2 thymocytes against C57BL/10 cells.

Results of this study suggest the presence in mouse serum of a factor (or factors) which confers a state of immunocompetence on a class of cell which resides in the thymus of young mice and which is highly reactive against the products of H-2. The serum component permitting mouse thymocytes to respond against H-2 alloantigens is the subject of much speculation, as is the mode of action. Results of experiments showing the effects of serum from normal and from homozygous nude mice on the thymocyte responses to H-2 and non-H-2 antigens strongly supports the concept that this is a 'thymus factor'.

At present, it seems most probable that mouse serum acts on the T-cell precursors or immature T cells of the thymus and induces the state of immunocompetence (for example, development of specific receptor sites on the surface of the T cell). This possibility is supported by studies²⁰ in which a serum factor secreted by humans and mouse thymus confers on immature cell populations rosette forming capacity or a state of responsiveness to concanavalin A (con A).

In addition to H-2 reactive cells, the thymus also contains a class of cell reactive against products of the non-H-2 loci. On the basis of their MLC responsiveness in the serum-free medium these are probably a different class of cell. It is unlikely that responses against non-H-2 alloantigens in serum-free medium are due to their being more immunogenic than the H-2 alloantigens, because in the serum supplemented medium responses to H-2 are usually stronger than to non-H-2 antigens (Fig. 1).

H-2 alloantigens represent a special class of antigens to the immune system—they serve as highly effective target antigens for T-cell mediated cytotoxicity whereas non-H-2 antigens do not⁸. Other studies (A. B. Peck, unpublished data) show that thymocytes develop cytotoxic potential after their strong MLC activation by H-2 alloantigens only in the presence of mouse serum; no CML is observed in serum-free medium. Thymocytes also fail to develop cytotoxic potential against non-H-2 alloantigens despite strong MLC activation in strain combinations possessing genetic differences at only non-H-2 loci in either medium.

This culture system may define two classes of T cells by their *in vitro* response patterns to H-2 and non-H-2 antigens, and provides an easy method for identifying T cell functions of the

two classes. Studies are in progress to separate and isolate the two cell types.

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Erratum

In the article "Science and works of art" by J. P. Brommelle and N. S. Brommelle (*Nature*, **250**, 767; 1974) the address given for N. S. Brommelle was incorrect. N. S. Brommelle is at the Victoria and Albert Museum.

Corrigendum

In the article "Radiological mapping of the ribosomal RNA transcription unit in *E. coli*" by P. B. Hackett and W. Sauerbier (*Nature*, **251**, 639; 1974) the authors erroneously referred to the work of Bleyman *et al.* (*J. Bact.*, **99**, 535; 1969) (ref. 20) as being conducted in *E. coli*. These studies of Bleyman *et al.* utilised *B. subtilis* in which the transcriptional order of the ribosomal RNA genes seems to be the same as in *E. coli* (Pace, N. R., *Bact. Rev.*, **37**, 562; 1973) (ref. 1).

matters arising

Immunopathy of parasitic infection

THE News and Views article 'Immunopathology of Parasitic Infection' by our correspondent F. E. G. C. (246, 187; 1973) has elicited several letters. He wrote "most parasites are capable of evoking immune responses in their hosts but these are seldom effective in eliminating the infection" and in conclusion that "as more information about immunity to parasitic diseases accumulates the possibilities of developing useful methods of immunisation fade further and further into the distance".

● Professor G. M. Urquhart of the University of Glasgow writes: "The first statement is inaccurate and the second is surely a personal opinion largely based on an erroneous extrapolation of the excellent work of Warren¹ who has shown that schistosome egg granulomata are the product of a delayed-type hypersensitivity reaction.

The development of a highly effective degree of acquired immunity both in the sense of the elimination of infection and in resistance to reinfection has been shown to occur in many experimental helminth infections and in domestic animals its natural acquisition plays an essential role in the survival and health of adult cattle, sheep and horses constantly exposed to heavy infection. Over the past 16 yr millions of calves in Europe have been successfully immunised with a vaccine prepared from X-irradiated larvae against *Dicrocoelium viviparus*, the lungworm of cattle.

The same situation applies to protozoal infections. Antigenic variation does present a problem in artificial immunisation but under natural conditions man and animals survive in large numbers in malarial and trypanosome endemic areas respectively, apparently through the development of an effective immunity. Leishmaniasis in experimental animals and man and coccidiosis in domestic animals also offer excellent examples in which acquired immunity is highly important in protection.

For many years immunological research on many of these important diseases was neglected and we should not be too dismayed if the present in-

terest in parasitic immunology elicits some facts which, at least at first sight, appear disheartening from the viewpoint of the rapid development of a comprehensive range of vaccines."

● Dr D. G. Colley of Vanderbilt University, Tennessee writes: "The final paragraph draws a conclusion regarding immunisation potentials in parasitic infections which, although possibly correct, is based on what I consider to be erroneous reasoning. It reflects a misinterpretation of the basic tenets of either parasitology or immunology, or both. I do not argue with the possibility that the induction of a protective immune response in schistosomiasis may be difficult, or even impossible to achieve. As has recently been pointed out such immune resistance mechanisms have certainly not been convincingly demonstrated and may not even exist.

The article seems, however, to base this assumption upon the demonstrated immunopathogenic effects of an anti-egg response. This ignores both the uniqueness of the various intramammalian stages of the schistosomes (schistosomules, adult worms, eggs) and the fundamental concept of immunological specificity. Certainly to induce or increase anti-egg cell-mediated responses could be potentially disastrous (although the induction of enhancing antibodies might be beneficial). Responses against egg antigens have, however, long been seen as of little protective consequence. What the discipline needs, and many investigators are pursuing, is more attention to immune responses against specific antigens."

● Dr T. A. Miller, of the Jensen-Salsbery Laboratories, Kansas City, Missouri, writes: "The article should more appropriately have been entitled 'Immunopathology of Schistosomiasis'. The emphasis on this one host-parasite relationship excludes, for all practical purposes, consideration of the other 95% of parasitic infection. Moreover, the final statement represents only one aspect of the current international opinion, viz-à-viz schistosomiasis.

As a general statement applicable to the other 95% of parasitic infections, this is patently uninformed and misleading in view of the proven efficacy, safety and widespread use on two con-

tinents of irradiated nematode vaccines for lungworm disease of cattle and sheep and hookworm disease of the dog."

¹ Warren, K. S., *Trans. R. Soc. trop. Med. Hyg.*, 66, 417 (1972).

Population estimates from recapture studies

BELL¹ suggested that $P = ((N-a)!(N-n)!)/(N!(N-a-n)!)$ is the probability that a population is not larger than N in size, where a animals have been captured, marked, and released, and on a subsequent occasion n animals have been captured, all of which have been found to be unmarked. Though P is indeed the probability of the latter event given that the size of the population is N , Bell advances no reason why the probability that the population is not larger than N should be equated to it. Indeed, Fisher² has stressed it should not: 'The direct step from the test of significance to a probability distribution cannot be sustained, and this circumstance has been responsible for some misunderstanding, and confusion of the terminology.'

Were Bell's treatment valid we could find the probability that the population is of size N exactly by subtraction, yielding

$$p = an \times ((N-1-a)!(N-1-n)!)/(N!(N-a-n)!)$$

Suppose one marked animal were released ($a = 1$) and subsequently one animal captured and found to be unmarked ($n = 1$). Then $p = 1/N(N-1)$, indicating, for example, that $N = 10$ is 110 times more probable than $N = 100$. But most people, I suggest, would feel that they had learnt very little about N (except that it was at least 2), and they would be almost indifferent between $N = 10$ and $N = 100$.

This is reflected in the solution given by likelihood theory³, in the context of which the problem is entirely standard. Since P is the probability of what was observed, given N , it is the likelihood of the hypothesis N given what was observed. It attaches a likelihood to each possible value of N , increasing from $n!a!/(n+a)!$ for $N = a+n$, to unity for N very large. For the case $a = n = 1$, $P = (N-1)/N$, indicating that 100 is only 1.1 times more likely than 10.

A minimum estimate of population size is given by the lower 2-unit support limit³ found by solving $P = 1/e^2 = 0.1353$ for N . By the very nature of the problem there can be no upper limit, and likelihood theory prescribes none.

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¹ Bell, G., *Nature*, **248**, 616 (1974).

² Fisher, R. A., *Statistical Methods and Scientific Inference* (Oliver and Boyd, Edinburgh, 1956).

³ Edwards, A. W. F., *Likelihood* (Cambridge University Press, 1972).

DR BELL REPLIES—Edwards makes two major points: first, that my estimate¹ of population size is strictly invalid; and secondly, that it is of no practical value in situations where its use might be considered.

Although no statistician, I have satisfied myself that Edwards' first point is correct, and that the closing sentences of my original communication must be revised. If no recaptures are made, then clearly $P = 1$ when the population is assumed to be infinitely large. This does not mean that it is absolutely certain that the population is infinitely large; on the contrary, we may be quite sure that it is not. It means that if the population were infinitely large, we could be quite certain that no recaptures would be made. At some finite value of N , say N^* , the value of P will be 0.5; that is, if we performed many recapture surveys, obtaining the same sample size on each occasion, we would fail to make any recaptures on about half the total number of occasions. If, therefore, we perform a recapture survey and obtain no recaptures, we still have no evidence that the population is really larger than N^* ; and it is in this sense that N^* is a minimum estimate of N . The value of P chosen depends on how certain we wish to be in setting an upper limit to N^* .

Edwards' second point seems less fully justifiable. The example that he gives involves the very smallest sample that can yield any information whatsoever concerning the size of the population, and it is not surprising that only the most tentative conclusions can be formed. With larger samples, useful information can be obtained which is not utilised by standard recapture techniques: in a study of the distribution of population size in newts (G.B., unpublished), the results obtained from the nil-recapture method were found to be entirely consistent with those obtained from two other techniques; stochastic and deterministic recapture surveys, and trap-ratio estimates (Fig. 1). Statistical analysis is primarily a means of interpreting observations of nature, rather than an independent academic discipline, and it is to be hoped that the nil-recapture

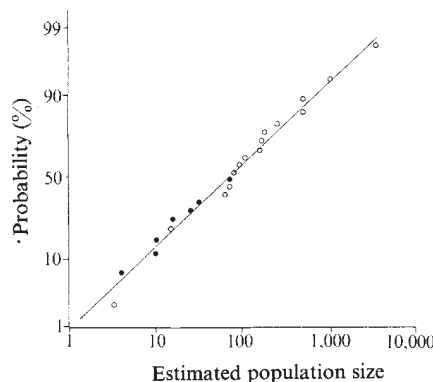


Fig. 1 The distribution of population size in the smooth newt (*Triturus vulgaris* (Linn)) near Oxford (G.B. unpublished). Each point represents the estimate of the size of a single population. The data are plotted according to the method of Harding² for small samples; population size can be seen to be log-normally distributed. ●, Estimates due to the nil-recapture method, using $P=0.5$; ○, estimates from standard recapture techniques, or from a known relationship between the rate of trap-captures and the total population size.

method will be useful, in some circumstances, to biologists working in the field. It may be that its rationale is less rigorous than that of the higher reaches of likelihood theory, but it is at least comprehensible to field biologists without specialised mathematical training. To accept mathematical conclusions whose truth one is unable to establish personally, is to accept the argument from authority—against which all scientific enquiry is a revolt.

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¹ Bell, G., *Nature*, **248**, 616 (1974).

² Harding, J. P., *J. mar. biol. Ass., U.K.*, **28**, 141–153 (1949).

Protein phosphorylation during oocyte maturation

Morrill and Murphy¹ suggested that “the release of prophase block at ovulation is associated with intense protein phosphorylation” coinciding with “the activation of a protein kinase (possibly *via* cyclic AMP)”. They further found that “the principal protein species phosphorylated between meiotic prophase and the second metaphase appears to be the phosphoprotein phosvitin”. But proper controls for their experiments seem to have been missing. It was not determined whether oocytes that remained in prophase block but were exposed to the same amount of $^{32}\text{P}_i$ for the same time as those undergoing maturation would also have phosphorylated endogenous protein, as our experience indicates^{2,3}. Nor is it clear that their phosvitin was authentic.

We have repeated their experiments, using an *in vitro* system. Approximately

120 fully grown oocytes from *Xenopus laevis* (injected with 1,000 U of human chorionic gonadotrophin 3 weeks previously to flush out over-ripe oocytes) were dissected manually from their follicles and placed for 2 h in 10 ml of solution O-R2 (ref. 4) containing 1 mCi $^{32}\text{P}_i$ (carrier-free, Schwarz/Mann). After 2 h they were washed, and half were placed in 15 ml of solution O-R2 containing 10 μg progesterone ml^{-1} to induce maturation⁵. Remaining oocytes were placed in progesterone-free medium (zero time). After 30 min both groups were transferred to medium lacking progesterone. At 6 h, approximately 60% of progesterone-treated oocytes had a diffuse white area centred at the animal pole, indicative of germinal vesicle breakdown⁶. By 9 h, virtually all treated oocytes had the white area, while untreated oocytes were negative. Dissection of boiled oocytes indicated that the appearance of a white area corresponded with the vesicle breakdown in every case examined ($N = 24$). Sterile technique was used, 10 μl of antibiotic-antimycotic solution (Grand Island Biological Co.) was added per ml of incubation medium, and the temperature was 20°C.

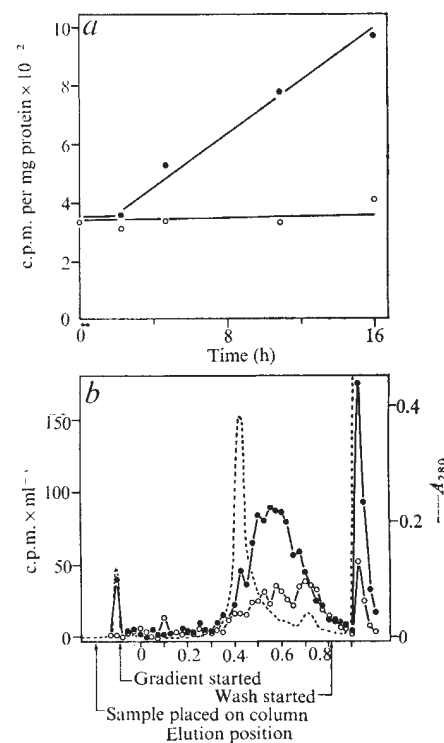


Fig. 1 *a*, Specific activity of ^{32}P -protein in progesterone-treated oocytes (●) undergoing maturation and control oocytes (○). Oocytes were preincubated for 2 h before zero time in $^{32}\text{P}_i$. Each point represents the average of three determinations. The arrow indicates the time of progesterone treatment. *b*, DEAE-cellulose chromatography of extracts from oocytes taken at the end of the 16-h period indicated in (*a*). The dashed line is the absorbance trace; other symbols are as above.

At various times oocytes were placed in water at 100° C. After several minutes, each was transferred to 0.5 ml of 1% sodium dodecyl sulphate plus 5 mM dithiothreitol (pH 8.0) and incubated at 50° C until dissolved. Samples of dissolved oocytes were placed on 23-mm diameter disks of Whatman No. 42 paper, air-dried and treated with cold and hot trichloroacetic acid (containing 1 mM NaH₂PO₄) and alcohol and ether to remove low-molecular-weight material, nucleic acid and lipid². The disks were counted for ³²P-protein in a Beckman Lobeta II planchet counter and then used to determine total protein⁶. Figure 1a shows that, after a lag of several hours, the specific activity of the progesterone-treated oocytes increased with time, whereas that of the untreated oocytes did not.

After 16 h, 45 oocytes from each group were homogenised in strong salt solution², dialysed and chromatographed on a 23/0.9 cm TEAE-cellulose column⁷. Under these conditions lipovitellin and phosvitin eluted at positions 0.42 and 0.72, respectively. Samples from each fraction were assayed for ³²P-protein³ (Fig. 1b). Neither lipovitellin nor phosvitin appeared to be specifically labelled in either case, and the increased amount of labelled material in progesterone-treated oocytes was found (a) initially percolating through the column, (b) between the lipovitellin and phosvitin peaks, and (c) eluting with the wash solution at the end of the run.

We conclude that there is an increase (although not intense) in protein phosphorylation during oocyte maturation, but that phosvitin is not involved. Phosvitin could serve as an energy source during embryogenesis, as suggested^{1,8}. The dephosphorylation observed by Morrill and Murphy during early embryogenesis, however, probably involves some other protein(s). We do not yet know whether the increased phosphorylation during oocyte maturation results from an "activation of a protein kinase (possibly via cyclic AMP)." We have previously described a protein kinase from amphibian oocytes and eggs that is stimulated by cyclic AMP when histone is used as the substrate but not when phosvitin is the substrate⁹.

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DR MORRILL REPLIES—Dr Wallace appears to confirm our observation¹ that release of the prophase block in amphibian eggs is associated with increased protein phosphorylation. The *in vitro* studies of Wallace and our *in vivo* studies are not, however, directly comparable. We injected ³²Pi directly into gravid females together with inducing levels of pituitary extract. It is our experience that isolated body cavity (ovulated) eggs take up and incorporate ³²Pi and would probably do so in both the body cavity and oviduct. The *in vivo* maturing eggs were thus exposed to ³²Pi for at least 24 h until released from the oviduct. In contrast, Wallace preincubated isolated oocytes with ³²Pi for 2 h and then transferred the oocytes to medium containing progesterone and unlabelled Pi. We have found that intracellular ³²Pi of excised oocytes exchanges rapidly with extracellular Pi. Thus, during the progesterone stimulus ³²P incorporation into protein would be limited to diminishing endogenous supplies of phosphorylated nucleotide. This might contribute to the lower overall protein phosphorylation reported in the *in vitro* system, and possibly to differences in the protein species phosphorylated.

The main difference between our results and those of Wallace is the identification of the phosphorylated protein species. A comparison of a number of phosvitin extraction methods indicated that the major ³²P-labelled protein(s) of the *in vivo* ovulated frog egg could be isolated by the method of Mano and Lipmann². This ovulated egg 'phosvitin' co-electrophoresed with commercial hen's (ovulated, unfertilised) egg phosvitin on SDS polyacrylamide gels. This 'phosvitin' differs from that characterised by Wallace³ in that: (1) it has a lower phosphate content; (2) it is extractable with lower salt concentrations; and (3) it is associated with the cortical pigment granule fraction and not with the yolk platelets. This suggests that amphibian egg phosvitin exists more than one metabolically active form, one principal difference being the level of phosphorylation. In this regard, Mano and Lipmann² have shown that fish egg phosvitin can be separated into discrete peaks containing 3.2, 5.7, 7.2, and 9.5% P. The existence of discrete phosphorylation levels of the same protein backbone structure is particularly interesting.

Neither our maturing oocytes nor Wallace's were compared with the ideal control, that is oocytes completely free of follicle cells. In contrast to the ovulated eggs used in our experiments, oocytes dissected manually as described by Wallace retain a surface layer of intact follicle cells and these cells may also actively phosphorylate protein. When we injected ³²Pi into gravid females with no hormone stimulation we recovered ³²P-labelled protein in excised ovaries and manually dissected oocytes after 24–48 h *in vivo*. But, there was no significant ³²P incorporation into the 'phosvitin' fraction isolated as described above. This latter information was accidentally omitted from our final reduced manuscript, through the oversight of the authors.

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Tin mineralisation

SILLITOE'S recent letter¹ on tin mineralisation and mantle hotspots is grossly misinformed about the age of the tin mineralisation in South West Africa. The tin deposits do occur in pegmatites but they are unrelated to the possible plume generated Jurassic-Cretaceous alkaline complexes in Damaraland. Instead the pegmatites are related to the Late Precambrian Damara orogen. Radiometric age determinations for a number of pegmatites are in the range 450 to 650 Myr (ref. 2.) I know of no tin mineralisation associated with the Mesozoic alkaline granites in South West Africa and they cannot, therefore, be used to support the contention of a relationship between tin-bearing silicic rocks and mantle hot spots.

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Primary structure of *E. coli* dihydrofolate reductase

In a recent paper¹, the primary structure of *Escherichia coli* dihydrofolate reductase was reported from this laboratory. The paper also suggested a

similarity between a portion of its primary structure and a portion of four vertebrate dehydrogenases.

In a private communication, Drs M. Rossmann and C. Brändén have informed us that the portions of three of the four dehydrogenases chosen for comparison are not from comparable areas of their tertiary structure²⁻⁴, and that the statistical treatment given in my paper is inappropriate.

The misalignment of the dehydrogenases does not, of course, affect the primary structure of dihydrofolate reductase shown in Fig. 1 of ref. 1.

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All female butterfly broods

OWEN, Owen and Chanter¹ have invoked Y-linked meiotic drive to explain the high frequency occurrence of all-female broods in some populations of the African butterfly *Acraea encedon*. This mechanism does not account for the following observations² of these populations: first, that they only occur in 'disturbed' areas; second, that females aggregate and lay egg batches in close proximity to each other; and third, that females 'court' other females.

The following explanations are advanced for these observations. First, an environmental disturbance (with its consequent effect upon larval food plants) will alter the spatial relationship between egg batches. In mixed sex progeny this may well increase the probability of the first matings being between brother and sister. All-female

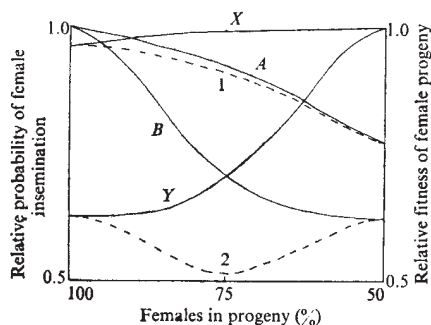


Fig. 1 Net female fitness values (curves 1 and 2) of broods varying in their sex ratio. Curves A and B show a reduction in fitness of females from mixed broods relative to that of all-female broods (as a result of inbreeding). Curves X and Y show the consequent reduction of female insemination probability in all-female broods relative to mixed broods. Net female fitness values calculated by multiplying relative probability of insemination of female progeny by relative fitness of female progeny (curve 1 = $A \times X$, curve 2 = $B \times Y$).

broods do not sustain this disadvantage. Such inbreeding will increase the homozygosity of the genetic environment surrounding the normal Y chromosome and viability loss is a well documented aspect of homozygosity³⁻⁵. Therefore the mutant chromosome will sustain an advantage and spread through the population. As it does so, the probability of all-female broods being laid near mixed broods increases and with it the inbreeding probability of these latter broods.

As male frequency drops, however, mixed brood females have a greater probability of insemination¹. The figure shows that with increased inbreeding the net fitness of all-female broods increases (curve 1) and then reaches equality (curve 2) with respect to mixed broods as male frequency drops.

Second, a mutant female laying its eggs in close proximity to a mixed sex batch will increase the insemination probability of its offspring. The aggregation and egg laying strategies may therefore be accounted for.

Third, any mutant female able to predict oviposition in another female and act as in the second case above will sustain an advantage. This, I suggest, may account for intra female 'court-ing'.

In non-disturbed (and possibly some disturbed) areas the Y mutant must sustain a net selective disadvantage as evidenced by its non-appearance. The nature of this selective pressure is not yet known.

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DR CHANTER REPLIES—Over the years, we have received many suggestions as to how the curious population biology of *Acraea encedon* might be explained, and we welcome another. There are, however, some difficulties with Manning's explanation of the tendency for the occurrence of all-female broods to be associated with 'disturbed' areas. Here I will confine myself to two of these difficulties, both concerned with the figure.

First, the lines on the graph are quite arbitrary, and the different slopes of curves 1 and 2 result from the fact that while curves Y and B have been drawn as reflections of one another (about a vertical line through the 75% point), X and A have been drawn asymmetrically. It would be easy to alter the asymmetry of curves X and A and reach a different conclusion.

Second, to obtain the net fitness of all-female broods relative to that of females from mixed broods (curves 1 and 2), it is necessary to multiply curves X and Y by the reciprocals of curves A and B rather than by curves A and B as they stand, since the relativity in curves A and B is opposite to that in X and Y.

Manning's explanation of the aggregating behaviour is interesting, but would be more convincing if the eggs laid by participating females were fertile—all the eggs we have collected from aggregations have been infertile¹.

Finally, I must emphasise that we have not established conclusively that the sex ratio trait is caused by a Y-linked gene, and that we also have some idea as to why the trait is not established in some populations, as explained in our paper² in which the peculiarities of an isolated population at Gegbwema in Sierra Leone are described. More recently we have discovered a population at Dar es Salaam in which the trait may be just beginning to appear and we hope that this discovery may yield additional information.

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reviews

Towards a new technology

R. G. Rhodes

THE publication of this book comes at an opportune time in the development of potentially important applications of superconductivity; a time when important decisions on the extension of existing programmes are under consideration.

Unlike the semiconductors, the future of superconductors seems to be in their application to large scale engineering devices such as high field magnets in particle accelerators, power cables, large machines and generators, and magnetic levitation and linear motor propulsion systems. The basic thinking behind nearly all of these schemes is that they apparently offer the best solutions to many of the economical and social problems facing the advanced countries of the west in such fields as the energy crisis, dwindling natural resources, and pollution and the environment.

Based on discussions that took place in a select NATO gathering of some of the world's leading experts in the fields of superconductivity and low temperature engineering, this book has been biased towards the large scale engineering projects rather than the purely scientific interests. The original conception and subsequent planning of the NATO Institute, which culminated in this publication, resulted almost entirely from the efforts of Simon Foner and Brian Schwartz of the Francis Bitter National Magnet Laboratory at MIT, who must be warmly congratulated.

Although the phenomenon of superconductivity was discovered by Kamerlingh Onnes as long ago as 1911, until recently it has been extremely slow in making its mark in the real world of practical technology. Only since our understanding of superconducting behaviour and the metallurgy of the type II materials has been established in the last decade or so has the successful manufacture of superconductors been possible. But although operation at liquid helium temperatures has become an engineering routine the real problem still remains, namely, that of introducing the conventional engineer to a new low-temperature environment and convincing him of the reliability and safety of cryogenic devices. This situation has been further aggravated by the huge development costs which inevitably are involved in all such large scale applications. The timely appearance of this book presents the situation

Superconducting Machines and Devices: Large Systems Applications. Edited by Simon Foner, and B. B. Schwartz. Pp. xx+692. (NATO Advanced Study Institutes Series.) (Plenum: New York and London; NATO Scientific Affairs Division, 1974.) \$47.00.

in exacting detail as it existed in seven different countries up to the end of 1973. It is divided into 17 chapters extending to some 690 pages, contributed by nearly two dozen authors.

The first seven chapters constitute by far the major contribution. Each treats in considerable detail a specific area of technology in which superconductivity is the essential part. The more significant fields of investigation are introduced in the first chapter by J. Powell of the Brookhaven National Laboratory. In particular, he makes a special case for the magnetic levitation of high speed ground transportation systems using the so-called null flux system, an area of applied superconductivity in which he has a special interest. The theory and fabrication of practical superconducting materials are discussed in detail in chapter 2. On the basis of our present metallurgical understanding and experimental research a T_c of 25 K could be realised by 1985. That would truly be a significant breakthrough for cryogenics.

The one important application in which superconductivity has found a permanent niche is that of high field magnets and, more particularly, in the field of high energy physics. The design, construction and behaviour of the more significant magnet systems in the various major laboratories throughout the world are discussed in chapter 3. The following two chapters are devoted to rotating machines. The design details of a superconducting homopolar motor, and its successful application at the Fawley power station, is described. From the results of further experimental projects on d.c. machines it is predicted that the heavy industrial power industry is poised on the threshold of a new era.

Considerable progress has also been made on parallel programmes investi-

gating the application of superconducting windings to a.c. machines, which are discussed at length in chapter 5. The preliminary design calculations indicate that the current density available with high field superconductors may well provide an increase in the economic power density by a factor of 5 to 10 over conventional machines, and may also have a significant volume advantage.

The case made in the first chapter for a mass ground transportation system using superconducting magnets is taken up again in chapter 6, with arguments for an alternative repulsion system and linear synchronous motor propulsion. From exhaustive theoretical studies confirmed by laboratory tests there seem to be no fundamental technical problems which remain to be solved. There are, however, many technical innovations needed before such a high speed transportation system can be put into practical use.

One of the first applications to be seriously examined was the superconducting power cable. With the constantly increasing demand for electrical power, generating stations of ever increasing size are required and, therefore, so are transmission systems of higher capacity and efficiency. The construction and configuration of both a.c. and d.c. cables are fully described in chapter 7. The 12 major superconducting cable projects being undertaken at 10 different laboratories throughout the world are considered to be fully justified by the fact that they represent the most economical solution for power ratings of 2,000 MW or more, which are expected to be the norm in the not too distant future.

Chapters 8, 9 and 10 are supplementary chapters in which three promising areas in the new technology are discussed. The use of hydrogen as an energy carrier is described in chapter 8, followed in chapter 9 by an account of the Josephson junction as a computer device. In chapter 10 a project investigating the use of superconducting magnets for high grade magnetic separation is outlined.

Finally, in seven relatively short chapters from 11 to 17, the essential details of the national programmes in applied superconductivity being undertaken in France, Germany, Italy, Japan, Switzerland, the UK and the US are outlined and details of the institutions, the personnel, and funding

have been included. That innovation in a book of this nature is a welcome addition and, as pointed out in the preface, should lead to better national planning as well as to international cooperation.

This is a book of international status in every sense and there seems little doubt that it should go a long way in promoting a better and wider understanding of this important area of low temperature technology. It should prove to be an invaluable aid to the decision makers in the assessment of future large scale technologies. □

Starting with an open mind

Development in Infancy. (A series of Books in Psychology.) By T. G. R. Bower. Pp. viii+258. (W. H. Freeman: San Francisco and Reading, July 1974.) £5.20 cloth; £2.90 paper.

It was the style of 18th Century philosophers of mind to premise their arguments upon speculative assumptions concerning the original nature and subsequent development of human perception. Bower's splendid little book is in that tradition—but with one hugely crucial difference. In place of speculative assumptions about the original nature of perception the pages of this volume provide a stunning array of experimental research, much of it by the author and his collaborators, designed to explicate how in fact the infant during the opening years of life comes to perceive space, objects, locus and trajectory, and how he develops appropriate concepts and behaviours to cope with what he comes to know. Bower has given us not only a compendium of empirical findings, but a speculatively (sometimes too speculatively) argued treatise on the starting nature of human knowledge and the course of its early transformation in response to encounters with the environment.

It may well be the most important work on early human development since the launching of Piaget's magisterial research a quarter of a century ago, and although it brings that work into question both broadly and in detail, it builds upon it as a starting point. For it is not the work of the Geneva group that is brought most deeply into doubt by this book, but rather the empiricist view of knowledge—the assertion that knowledge is acquired by the gradual accretion of associations or stimulus—response connections that mirror the state of the environment. Indeed, Bower's conclusions point instead to the early importance of man's inherent structure, to the array of

species-typical readiesses to register upon and organise knowledge by highly ordered rules reflecting man's evolution.

Development goes forward by stages of organisation, each stage supplanted by a later stage when usable conflict occurs in the attempted application of earlier rules. It is the concept of usable conflict that distinguishes the author's view from that of conventional learning theory, for encounters with the environment are shown to be effective in producing change only when the infant is able to code the disparity between his expectancies and what actually transpires as a result of his responding. The codability of such mismatches depends upon the development of rule structures for interpretation—the result of maturation shaped by experience.

For Bower, the initial structure of space for the infant is supramodal, a space into which the special senses are mapped. Sufficient experimental evidence is presented to raise sharp questions about both Berkeley's and Piaget's accounts of the associative or conceptual welding of the disparate spaces of eye, ear, and hand. New and searching techniques of research are now available—many of them invented by the author—that suggest that the hand knows what the eye knows before it has had its own experience of reaching under visual control. "It is highly improbable", he argues, "that any organism evolved a visual system or an auditory system that was not an outgrowth of the supramodal perceptual system . . . the system that responds to those properties of objects that can be specified via any sensory modality." In like vein, primitive visual objects, although they are elaborated by 'experience' simultaneously to encompass more properties, begin as inherently organised representations including more than a single modality. Witness the distressed surprise of the infant at the beginning of his manipulatory career who reaches out for a virtual object produced by polarised light and polarising spectacles, and finds no tactual feedback on grasping the target. An older child is less surprised, having come to differentiate palpability from visual object qualities.

Bower then argues that at the earliest stage, as indicated by studies of eye-movement and reaching, the infant has two disparate systems for identifying and conserving the identity of objects. One is for objects in motion and is based on a smooth pursuit system; the other is for static objects and is based on the saccadic system. Only with maturation and with appropriate experience, Bower argues, do infants develop rules for dealing simultaneously with the two systems.

As far as cognitive development is concerned, it is conceived of as the development of rules and regulatory processes that serve to organise different features of input and to sequence classes of behaviour rather than specific behaviours. Bower argues that no specific single behaviour is ever found to be crucial to development; adequate 'manipulatory' behaviour seems to develop even in limbless thalidomide children who carry out reaching and prehensive tasks with their mouths and teeth. "It is more parsimonious to assume change in an underlying concept which generates all of the behaviours, than to assume separate independent changes in each behaviour." Cognitive development, then, is seen as the progressive growth of increasingly comprehensive and differentiated control systems for constructing and sequencing behaviour. Bower presents a brilliant series of experiments to illustrate his point, centred upon the development of the concept of an object; how it is recognised as the same object though undergoing transformations in appearance, locus, and even singleness of identity.

But for all its virtues and power, this book is flawed in a way that destines it to criticism and challenge. It is flawed, ironically, by an overextension of its virtues; its very clarity of premise and argument, capped by uncompromising conclusions, makes it vulnerable. It will serve for years as a source of ideas for doctoral theses that will prove it inconclusive theoretically and wrong or partially wrong experimentally. For, in fact, there are not yet enough findings in the field of infant cognitive research to justify the inferential depth of Bower's arguments or the boldness of his conclusions. And though the experiments—perhaps too closely focused on work at Edinburgh, Harvard, and Geneva—are highly ingenious, and often brilliant, many of them are neither easily reproducible nor yet as conclusive as the author believes. (Witness his reliance on a doubtful study by Wertheimer indicating that newborns can orient visually to a voice source in space; or the still questionable reproducibility of some of his own results.) Indeed, because of the clarity and depth of the premises, the crisply unqualified statement of the findings, and the forthrightness (at times recklessness) of conclusions, the book will provide both a paradigm for next stages of research—and a whipping boy.

Yet, however much it will be attacked as 'premature', it is a major synthesis of work on the "original nature of mind" and will rank as one of the major books on human development to be written during the last decade.

Jerome Bruner

Not so elementary

Introduction to Particle Production in Hadron Physics. By S. Humble. Pp. viii+253. (Academic Press: London and New York, 1974.) £6.80; \$17.50.

THERE are some subjects that cannot be taught. One can only learn them by experiencing their harsh realities oneself. And one can only face such a task when driven by powerful motivation or attracted by the hint of great excitement to be found along the thorny path. Just such a subject is particle production in hadronic interactions.

That the author of this book has succeeded in producing a clear and balanced exposition of an incredibly difficult topic, is noteworthy. That he has failed to generate in us the necessary desire and excitement is a pity.

It is an unhappy, but surely not an unexpected fact, that as elementary particles are made to collide at ever higher energies, more and more debris of an increasingly complicated character is found to emerge from the collision. It is by studying this debris that we are supposed to be able to deduce the structure and the dynamical properties of the colliding particles. But what a daunting task. Even a description of the debris (that is, of the momenta and energies of the emerging particles) is too complicated to comprehend. How do you communicate to someone your findings concerning the dependence of a scattering amplitude upon the 14 variables needed to characterise a final state of 6 particles?

It is well nigh impossible, therefore, to hope to work back from data to dynamics. Rather, one postulates a dynamical model and attempts to confront it with the data.

There are several models currently in vogue, all interesting, none very convincing. The author attempts to describe and compare them fairly comprehensively. I found the discussion of dual models very informative, especially the treatment of their predictions and of the difficulties involved in their computation. The section on the analytical properties of production amplitudes is particularly valuable, if only for the fact that it exposes the enormous complexity of the problems encountered. Multiperipheral and multiregge models are presented with a nice balance between essential fact and gory detail.

The text is marred by ugly typography (even an equation split between pages) and by many errors. I cannot refrain from seizing on the following sentence which most appropriately appears just below a typographical error: "Hence, it must always be a good idea in a practical calculation to

derive as much of the formalism as one can for oneself, rather than relying on even the most detailed texts."

Other points to carp at: too little physical motivation for the ideas of 'scaling' in inclusive reactions; too much emphasis on the original work of Lurcat and Mazur on phase-space integrals, when more modern and simpler techniques exist; a lack of depth and a certain banality in summarising the properties of the models.

All said, however, this is a useful book. It is a good attempt to come to grips with an enormously difficult subject. But—and this must be stressed—it is essentially a book for the would-be professional, and it demands more than a modicum of expertise in the theory of elementary particles. **E. Leader**



An early bird: the little owl, first introduced into Britain—where it is still relatively uncommon—during the last century. From *Birds*. By Christopher Perrins. Pp. 176. (Collins: London, September 1974.) £1.95. One of a series of books which also includes *Woodlands*, by William Condry, and *Life on the Sea Shore*, by John Barret.

Encompassing the North Atlantic

The Ocean Basins and Margins. (Vol. 2, *The North Atlantic*.) Edited by A. E. M. Nairn. Pp. xiv+598. (Plenum Press: New York and London, 1974.) \$45.60.

THE North Atlantic must be among the most comprehensively surveyed ocean basins; and in order to arrive at a picture of its age and evolution it is necessary to consider not only the marine geophysical data amassed in recent years, but also a century or

more of detailed geological research on the bordering continents. A few earth scientists are able to keep abreast of this vast volume of data. The rest of us, I imagine, tend to rely on the available reviews; and, ideally, these should be compiled in one authoritative and up-to-date volume covering every aspect of the problem. The fact that this second volume in the series *The Ocean Basins and Margins* comes close to realising the ideal should make it a much sought-after book.

Sins of omission are generally few, though chapters on the palaeomagnetic data from the North Atlantic continents and on sedimentation would have been welcome, and the northern reaches of the ocean deserve more attention. A general criticism which can be levelled at this volume, in its role as a source book, is that some chapters make reference to work published in 1973, whereas others, judging from their reference lists, were completed and on the editor's desk in 1971. There was surely ample time for the latter to be brought more up to date, if only by following the example given in M. J. Keen's chapter, which includes an appendix of useful references published after the completion of the manuscript.

On the credit side, however, the book does cover much the greater part of what needs to be known about the North Atlantic. The editors set the scene in their opening chapter with a qualified acceptance of the 1972 Pittman and Talwani model for the evolution of the ocean basin. Stehli and Keen review the geology of the well known western margin (Bahamas to Newfoundland) and point out some of the problems which remain to be solved. Owen (Western Approaches to the British Isles), Vigneaux (Biscay) and Dillon and Sougy cover the eastern margins, the last of these authors contributing a particularly valuable summary of western African geology. The major oceanic islands—Azores (Ridley, Watkins and MacFarlane), Canaries and Cape Verde (Dillon and Sougy) and Iceland (Noe-Nygaard)—are all described, the last in a good review of the North Atlantic Cainozoic volcanism. Evidence for an older 'proto-Atlantic' is to be found in the Lower Palaeozoic orogenic belts of the northern Appalachians (Williams, Kennedy and Neale), southern Caledonides (Dewey), and Scandinavia (Nicholson). The account of the tectonics of Newfoundland and the summary of the British Caledonides are particularly good. Additional references to the Caledonian orogeny, and much useful data pertaining to the early history of the ocean basin of the present northern North Atlantic can be found in a clear

account of the geology of Greenland's Atlantic coast. Finally, Fitch, Miller, Warrel and Williams bring together the literature on tectonic and radiometric age comparisons across the North Atlantic and Noltimier rounds off the volume with a comprehensive review of the geophysics of the ocean basin.

The book is well-produced, adequately illustrated, and there are extensive reference lists attached to each chapter. A subject index for the whole volume is provided, though a quick test of its usefulness revealed a "Silurian" entry which gives no page references for the Lower Palaeozoic orogens. The book contains the usual splattering of unimportant misprints, and one or two more serious gaffes, such as the omission of the decimal points from the seismic wave velocities in Fig. 16 (p. 425). It is already somewhat dated, notably in relation to the marine geophysical and deep-sea drilling data published in the last couple of years. That is, however, inevitable and, with the reservations already mentioned, of no great significance. The important thing is that anyone now contemplating a study of the North Atlantic and its environs can have at his elbow one volume providing much of the necessary geological and geophysical background to his work. Every earth science library should have a copy of this book.

R. J. Bailey

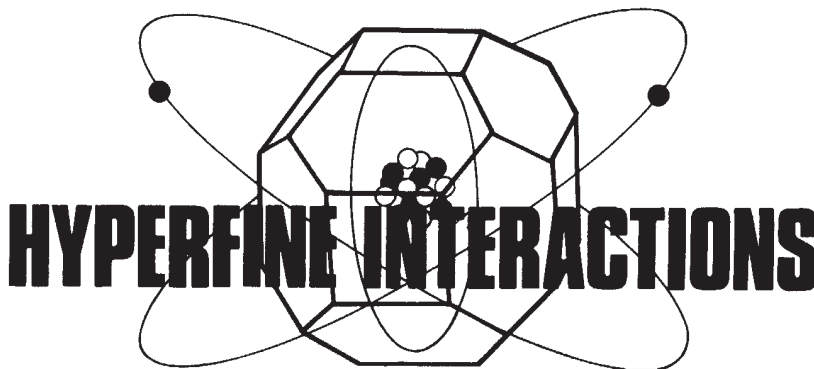
Liquid scintillation counting

Applications of Liquid Scintillation Counting. By D. L. Horrocks. Pp. xiii+346. (Academic Press: New York and London, June 1974.) \$29.50; £14.15.

LIQUID scintillation counting originated 25 years ago when Ageno and his coworkers discovered that organic liquid solutions, when exposed to ionising radiation, emit scintillations which may be detected by a photomultiplier. Dr Horrocks' book provides an account of factors involved in internal scintillation counting. It also discusses applications of the technique, including chemiluminescence and bioluminescence, Cerenkov counting, pulse shape discrimination, flow-cell counting and large-volume counting. The discussion of quench correction unfortunately omits such topics as the relative quenching susceptibility of different scintillators, the different effects of impurity (chemical) and colour quenching, and the relative efficacy of the various quench correction methods. Nevertheless, the book can be recommended as a useful source of information on the applications of liquid scintillation counting.

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HYPERFINE INTERACTIONS

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Scope

The journal is concerned with research in the border regions of solid-state, atomic, and nuclear physics. Although the title and subject **HYPERFINE INTERACTIONS** is one of the best examples of such a topic, other studies which utilize the modern techniques of atomic and nuclear physics may fall within the domain of the journal. The main concern is the understanding of border region physics, no matter what are the disguised varied forms in which it appears.

The hyperfine interaction Hamiltonian is the logical basis for a large part of the subject matter to which this journal will be devoted because it contains products of atomic and nuclear quantities. In condensed matter, the Hamiltonian even spans solid-state physics. Hence such fields as: perturbed angular correlations, nuclear magnetic resonance, electric quadrupole resonance, beam-foil spectroscopy, nuclear orientation, and Mössbauer effect studies fall immediately within the journals domain. In addition, any atomic or solid-state studies via hyperfine spectroscopy (utilizing mesons, gamma rays, electrons, etc.) are within the scope. It can include problems related to solid, liquid and gaseous states as well as studies with biological material.

The title **HYPERFINE INTERACTIONS** is not meant to prevent contributions that do not directly involve such interactions from reaching the journal. For example, the journal is receptive to particle-beam studies which also explore the border physics region but

do not necessarily utilize hyperfine effects. Under this category lie channeling, nuclear lifetimes studied via channeling and implantation phenomena. In fact, studies which incorporate one of the disciplines of physics: solid-state, atomic or nuclear physics to study another would be generally acceptable.

Advisory editorial board

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Invitation to authors

The editors cordially invite colleagues to submit papers for publication within the scope of the journal. It should be realised that the refereeing criteria will be high. Guidelines for preparing the manuscripts are available from the editors and publisher. Contributions should be sent to B. Deutsch, University of Aarhus.

Subscription information

The journal will be published in volumes of 6 issues; each issue will contain some 100 pages, first issue is scheduled to appear in spring 1975; In principle the journal will appear bimonthly. Subscription price: US\$42.50/Dfl.110.00 per volume, postage included. Specimen copies will be supplied by the publisher.

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